

10593387

File 5:Biosis Previews(R) 1926-2009/May W2
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Set	Items	Description
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? s	inulin and (reducing())sugar)	
	9586	INULIN
	167154	REDUCING
	120011	SUGAR
	3002	REDUCING(W) SUGAR
S1	9	INULIN AND (REDUCING()) SUGAR)
? s	inulin and tagatose	
	9586	INULIN
	373	TAGATOSE
S2	5	INULIN AND TAGATOSE
? s	inulin and glucose	
	9586	INULIN
	373620	GLUCOSE
S3	992	INULIN AND GLUCOSE
? s	inulin and (freeze or lyophil? or air)	
	9586	INULIN
	34298	FREEZE
	11482	LYOPHIL?
	213965	AIR
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
? s	inulin and hemoglobin	
	9586	INULIN
	89346	HEMOGLOBIN
S5	48	INULIN AND HEMOGLOBIN
? s	s5 and glucose	
	48	S5
	373620	GLUCOSE
S6	8	S5 AND GLUCOSE
? s	s5 and tagatose	
	48	S5
	373	TAGATOSE
S7	0	S5 AND TAGATOSE
? s	inulin and (peg?(3)hemoglobin)	
	9586	INULIN
	0	PEG?(3)HEMOGLOBIN
S8	0	INULIN AND (PEG?(3)HEMOGLOBIN)
? s	inulin and (peg?(3w)hemoglobin)	
	9586	INULIN
	27290	PEG?
	89346	HEMOGLOBIN
	81	PEG?(3W)HEMOGLOBIN
S9	0	INULIN AND (PEG?(3W)HEMOGLOBIN)
? ds		

Set	Items	Description
S1	9	INULIN AND (REDUCING()) SUGAR)
S2	5	INULIN AND TAGATOSE
S3	992	INULIN AND GLUCOSE
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
S5	48	INULIN AND HEMOGLOBIN

S6 8 S5 AND GLUCOSE
 S7 0 S5 AND TAGATOSE
 S8 0 INULIN AND (PEG?(3)HEMOGLOBIN)
 S9 0 INULIN AND (PEG?(3W)HEMOGLOBIN)

? s s1 and s4

9 S1

122 S4

S10 0 S1 AND S4

? s s4 and s5

122 S4

48 S5

S11 2 S4 AND S5

? t s11/7/1-2

11/7/1

DIALOG(R)File 5:Biosis Previews(R)

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13317004 BIOSIS NO.: 199698784837

Cross-linked %hemoglobin% increases fractional reabsorption and GFR in hypoxic isolated perfused rat kidneys

AUTHOR: Baines A D (Reprint); Christoff B; Wicks D; Wiffen D; Pliura D

AUTHOR ADDRESS: Dep. Clinical Biochemistry, Univ. Toronto, 100 College Street, Toronto, ON M5G 1L5, Canada**Canada

JOURNAL: American Journal of Physiology 269 (5 PART 2): pF628-F636 1995 1995

ISSN: 0002-9513

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: We compared the ability of human red blood cells (RBC) and a cell-free oxygen carrier to maintain isolated perfused kidney function under moderately hypoxic conditions. Recirculating perfusate was gassed initially with 93% %air%-7% CO-2, and, after 30 min, the gas was changed to 12 O-2-7 CO-2-81% N-2. Oxygen content of the perfusate was increased with RBC (30 g/l Hbg) or highly purified human %hemoglobin% A-0 (HbA-0) polymerized with O-raffinose (o-R-poly-Hb, 30 g/l Hbg). For comparison, kidneys were perfused with 60 g/l of bovine serum albumin (BSA) alone. The effects of unmodified %hemoglobin% were examined by adding 5 g/l of nonpolymerized HbA-0 to the BSA perfusate after 20 min. The effect of increasing oxygen delivery without %hemoglobin% was examined by switching to 93% O-2 after 20 min during some BSA perfusions (BSA-HiO-2). Vascular resistance decreased progressively in o-R-poly-Hb- and BSA-HiO-2-perfused kidneys but remained constant in other experiments. Nitro-L-arginine methyl ester (L-NAME) prevented vasodilation and increased the filtration fraction of o-R-poly-Hb-perfused kidneys with no change in other functions. L-NAME also prevented the formation of methemoglobin. After a 70-min perfusion with BSA, Na reabsorption was 82 +/- 3% (means +/- SD), and %inulin% clearance (glomerular filtration rate (GFR)) was 0.66 +/- 0.33 ml cntdot min-1 cntdot g-1. RBC increased reabsorption to 95% (85-98%) (median, 25th-75th percentile) but did not alter GFR (0.52 +/- 0.26 ml cntdot min-1 cntdot g-1). o-R-poly-Hb increased Na reabsorption proportionately more than GFR, so that, while GFR was doubled to 1.04 +/- 0.40 ml cntdot min-1 cntdot g-1, Na reabsorption increased to 98% (92-99.5%). HbA-0 increased GFR to 1.07 +/- 0.44 ml cntdot min-1 cntdot g-1 and increased

reabsorption to 89 +- 6%. A similar increase in Na reabsorption (93 +- 2%) and GFR (1.38 +- 0.3 ml cntdot min-1 cntdot g-1) was produced by increasing O-2 content of BSA with 93% O-2. o-R-poly-Hb was most effective in raising and maintaining overall renal function and lowering urine Na concentration and protein excretion.

11/7/2

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10306309 BIOSIS NO.: 199090090788

THE RETENTION OF ENTRAPPED MOLECULES WITHIN ERYTHROCYTE GHOSTS DURING
CRYOPRESERVATION

AUTHOR: BREARLEY C A (Reprint); HODGES N A; OLLIFF C J

AUTHOR ADDRESS: DEP PHARM, BRIGHTON POLYTECHNIC, LEWES RD, BRIGHTON BN2
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JOURNAL: Journal of Pharmacy and Pharmacology 42 (5): p297-301 1990

ISSN: 0022-3573

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: In view of the interest in erythrocyte ghosts and carrier erythrocytes as potential drug delivery systems, this work was undertaken to determine conditions facilitating the retention of entrapped molecules during cryopreservation. Upon %%%freeze%%-thaw treatment intact erythrocytes and erythrocyte ghosts displayed different damage profiles with respect to cryoprotectant concentration. Non-penetrating cryoprotectants showed optimum protection of intact cells at 0.cntdot.4-0.cntdot.5 M; this optimum was not observed with ghosts, in which damage decreased with concentration up to 1.cntdot.0 M. The concentration optimum for intact cells was not abolished by oxidative or reductive treatments suggesting that its absence in ghosts is not due to altered protein-protein-lipid interactions. The extent of %%%freeze%%-thaw damage to ghosts was influenced by the qualitative ionic composition of a cryoprotectant-free suspending medium, with 10-12% haemolysis observed in the presence of Li+ and Mg2+ but > 60% for Na+, Cs+, K+ and NH4+ with increasing loss following that order. The release on freezing of entrapped haemoglobin, %%%inulin%% and sucrose was found to be inversely proportional to their molecular weights.

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Set	Items	Description
S1	9	INULIN AND (REDUCING() SUGAR)
S2	5	INULIN AND TAGATOSE
S3	992	INULIN AND GLUCOSE
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
S5	48	INULIN AND HEMOGLOBIN
S6	8	S5 AND GLUCOSE
S7	0	S5 AND TAGATOSE
S8	0	INULIN AND (PEG?(3)HEMOGLOBIN)
S9	0	INULIN AND (PEG?(3W)HEMOGLOBIN)
S10	0	S1 AND S4
S11	2	S4 AND S5

? t s1/7/1-9

1/7/1

DIALOG(R)File 5:Biosis Previews(R)

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0019840702 BIOSIS NO.: 200700500443

Chemical composition and storability of Jerusalem artichoke tubers

AUTHOR: Barta J; Patkai Gy (Reprint)

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JOURNAL: Acta Alimentaria 36 (2): p257-267 JUN 2007 2007

ITEM IDENTIFIER: doi:10.1556/Aalim.36.2007.2.13

ISSN: 0139-3006

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Five different Jerusalem artichoke cultivars were investigated to compare their nutritional value. Investigations were carried out on samples harvested in December 2004 and stored until the end of March 2005 under natural climate in prism, in cold store and also after over-wintering in the soil. Investigations were repeated in 2005 and 2006. According to the results of storage outdoors in prism and in cold store, the total- and soluble solid content, the total carbohydrate and %inulin content (w/w) did not change significantly as a function of storage time. Changes in sucrose- and %reducing% %sugar% content and that of glucose/fructose ratio were also insignificant. There was no significant difference in the ratio of carbohydrates of the tubers stored under those two above-mentioned conditions, however, there was a significant difference in the carbohydrate composition of the tubers harvested in winter or in spring. Compared to the majority of vegetables, the main nutrient of Jerusalem artichoke tubers is %inulin%, instead of starch. Total carbohydrate content of the tubers is divided into 80-90% %inulin%, 7-14% sucrose and 3-6% reducing sugars, on average. Because of its high average yield and outstanding %inulin% content, this is a plant of great interest as raw material for %inulin% and fructose processing, as well. The cultivar "Cegledi" is, first of all, suggested for industrial processing. The present research data verified its outstanding %inulin% content and a high fructose/glucose ratio, too.

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16056977 BIOSIS NO.: 200100228816

Short photoperiods induce fructan accumulation and tuberous root development in Dahlia seedlings

AUTHOR: Legnani Garry (Reprint); Miller William B

AUTHOR ADDRESS: Department of Horticulture, Clemson University, Clemson,
SC, 19624, USA**USA

JOURNAL: New Phytologist 149 (3): p449-454 March, 2001 2001

MEDIUM: print

ISSN: 0028-646X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The effect is reported here of photoperiod on fructan accumulation in the tuberous roots of Dahlia sp. cv. Sunny Rose seedlings. Growth parameters were measured of shoots and roots on glasshouse-grown dahlia seedlings subjected to either short day (SD) or long day (LD; 4 h night photoperiod interruption) light regimes. The carbohydrate concentrations of tuberous roots was analysed by high performance anion exchange chromatography. Total plant dry weight was unaffected by photoperiod. The LD treatment inhibited tuberous root development but increased shoot dry weight. Tuberous root tissue of SD seedlings showed a 156% increase in total fructan (%%%inulin%%%) concentration compared with LD tuberous root tissue, which had higher %%%reducing%%% %%%sugar%%% concentrations than SD tuberous roots. A wide range of oligomers increased during the SD treatment. Sucrose appears to be the regulating factor in fructan metabolism in dahlia. Photoperiod is a valuable tool for studying fuctan metabolism in vivo, as it provides a nondestructive means of regulating sucrose partitioning.

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14015706 BIOSIS NO.: 199799649766

Fructan biosynthesis in excised leaves of Lolium temulentum. VII. Sucrose and fructan hydrolysis by a fructan-polymerizing enzyme preparation

AUTHOR: Cairns Andrew J (Reprint); Bonnett Graham D; Gallagher Joseph A; Simpson Richard J; Pollock Christopher J

AUTHOR ADDRESS: Cell Biol. Dep., Inst. Grassland Environmental Res., Plas Gogerddan, Aberystwyth SY23 3EB, UK**UK

JOURNAL: New Phytologist 136 (1): p61-72 1997 1997

ISSN: 0028-646X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A partially purified enzyme preparation from leaves of Lolium temulentum L. was previously shown to catalyse the net synthesis of oligofructans and polyfructans from sucrose. Here the same preparation is shown to catalyse the hydrolysis of both sucrose and oligofructans. The magnitude and properties of these hydrolytic activities have been determined. The significance of these catabolic activities for studies of fructan polymerization both in vitro and in tissues in a physiologically anabolic state are discussed. The preparation hydrolysed 1-kestose, 6-kestose, neokestose, %%%inulin%%% oligosaccharides of low degree of polymerization (DP 4 and 5) and endogenous oligofructans from L. temulentum, with the concomitant release of monosaccharide. The preparation also released %%%reducing%%% %%%sugar%%% at low rates from high molecular weight %%%inulin%%% but had no detectable activity against bacterial levan. Simultaneous incubation of sucrose and Neosugar (a commercially available mixture of predominantly beta-2, 1 linked tri-, tetra- and penta-saccharides) showed that sucrose was preferentially hydrolysed by the preparation, with Neosugar fructans being protected from hydrolysis at sucrose concentrations gt 30 mol m⁻³. The kinetic properties for hydrolysis of both sucrose and Neosugar were determined. For sucrose and Neosugar respectively, Michaelis constants at 30 degree C

and pH 6.0 were 7.7 ± 0.5 and 14.1 ± 1.1 mol m⁻³ (as terminal fructose) and maximum velocities were 6.5 ± 0.1 and 2.7 ± 0.1 mg g⁻¹ fr. wt h⁻¹ (equivalent to 10.0 and 4.2 nkat g⁻¹ as %reducing% %sugar% release). Maximal temperatures for activity were 45 and 44 degree C, and Arrhenius activation energies were 39.9 and 46.9 kJ mol⁻¹. Preincubations for 1h at 49 and 48 degree C caused 50% loss of activity in subsequent assays at 30 degree C. The pHs for maximal activity for the two substrates were 5.2 ± 0.1 and 5.5 ± 0.1 . Using size exclusion chromatography (SEC), an activity catalysing the formation of fructan oligosaccharides and another catalysing sucrose hydrolysis, were not fully resolved, but exhibited distinct profiles of elution indicating M-r, of 57 and 133 kD respectively. When assayed for the hydrolysis of Neosugar, the SEC eluate exhibited two peaks of activity indicative of M-r values of 57 and 133 kD.

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10265601 BIOSIS NO.: 199090050080

INFLUENCE OF VARIETY DATE OF HARVEST AND STORAGE TIME ON FACTORS CONNECTED WITH THE CRYSTALLIZATION ON CANNED SCORZONERA SCORZONERA-HISPANICA

AUTHOR: VULSTEKE G (Reprint); CALUS A

AUTHOR ADDRESS: PROVINCIAL ONAERZOEK, EN VOORLICHTINGSCENTRUM LAND-EN

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JOURNAL: Plant Foods for Human Nutrition (Dordrecht) 40 (2): p149-166 1990

ISSN: 0921-9668

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: In the period 1983-1986, research was carried out into the %inulin% content of scorzonera during ripening and storage. Since the %inulin% content is determining for the occurrence of crystallisation with canned scorzonera, the effects of the varieties, the time of harvest and storage of the scorzonera were investigated. The changing of the %inulin% content on the conversion into reducing sugars was checked; the effect of the dry matter and nitrate content were also defined. The aim was to define whether the determination of the %inulin% content was a useful parameter for the ripening of the scorzonera. On the whole, the different varieties showed remarkable differences where fructosanes + %inulin%, as well as pure %inulin%, were concerned. A significant decrease of the %inulin% content was obtained from the middle of November, by so far that it was below the limit above which crystallisation takes place. A two-month storage period of scorzonera harvested in early October also led to a very low %inulin% content, so that no problems could occur while canning. Storage conditions of the scorzonera seemed of importance too. The nitrate content of the different scorzonera varieties was low, although some variations were noted. The crops harvested in early December showed considerably lower contents when compared to those harvested in early October or mid-November. The %inulin% content as well as the content of %reducing% %sugar% s are a useful parameter to determine the maturity of the scorzonera.

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0001162878 BIOSIS NO.: 19613600072790

Ionic regulation in the crab *Carcinus maenas* in relation to the molting cycle

AUTHOR: ROBERTSON JAMES D

AUTHOR ADDRESS: U. Glasgow, Scotland

JOURNAL: COMP BIOCHEM PHYSIOL 1 p183-212 1960 1960

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Unspecified

ABSTRACT: The concns. of the principal ions Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺, Cl⁻, and SO₄⁻⁻, in the blood plasma of *C. maenas* have been compared with those of sea water in which the crabs were kept. Taking the total ionic concn. of the sea water as 100, the mean values with standard errors for the plasma at different stages of the molting cycle are as follows: intermolt 100.7 [plus or minus] 0.26, premolt 107.5 [plus or minus] 1.00, postmolt within 24 hrs. 102.9 [plus or minus] 0.56, postmolt 2 to 14 days 98.2 [plus or minus] 0.37, molted several months 100.3 [plus or minus] 1.35. Increases of Na, Ca, Mg and Cl are responsible for the rise in total concn. in the premolt stage; Ca increases from 126 to 164% of the sea water value, Mg from 37.1 to 55.5%. Measurements of total particle concn. by the Krogh-Blades method show the same pattern as the chem. estns. From the concns. of nonprotein amino-N, lactic acid, %reducing% %sugar%, NH₄, inorg. phosphate, and HCO₃ in the plasma, it is improbable that at any stage each of these constituents (except HCO₃) contributes 2-3 millimoles or mg. ions to the osmotic concn.; the latter is about 1080 mg. ions/kg, water at the intermolt stage. Uptake of ions and water in intermolt *C. maenas* takes place chiefly through the gills. Uptake of water at molt, averaging 66.3% (range 43-96%) of the premolt wt, takes place through the foregut and hepatopancreas. Chem. analyses show that the fluid absorbed is essentially sea water, with all its ions. Absorption of water into the extracellular fluid halves the concn. of blood protein. About 1/3 of the water is absorbed intracellularly. Extracellular vol. in intermolt crabs, as detd. by the distribution of injected sucrose and %inulin%, averages 32.6 ml. per 100 g. (30.2-34.9 ml. in 4 estns.). Hepatopancreatic secretion differs inorganically from plasma in having higher K, Ca, and Mg concns. During the postmolt phase all the ions of the secretion except Cl are higher than those of the plasma, SO₄ being 30 times as high. SO₄ accumulates owing to the slowness of its absorption compared with the other ions present in sea water. 51 references. ABSTRACT AUTHORS: Courtesy Chem. Absts

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0000805960 BIOSIS NO.: 19542800029718

The use of cation-exchange resins for the hydrolysis of sucrose in plant extracts

AUTHOR: NOGGLE G R

AUTHOR ADDRESS: Southern Res. Inst., Birmingham 5, Ala.

JOURNAL: PLANT PHYSIOL 28 ((4)): p736-740 1953 1953

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Unspecified

ABSTRACT: Sucrose was completely hydrolyzed in 40 min. at the temp, of boiling water when stirred with a synthetic sulfonic acid-type cation-exchange resin. The reducing sugars formed during the hydrolysis were determined by the Somogyi titration method. Dowex-50 (Dow Chemical Co., Midland, Mich.), Amberlites IR-100 and IR-120 (Rohm and Haas Co., Philadelphia, Pa.) and Duolite C-3 (Chemical Process Co., Redwood City, Cal.) were equally effective for the resin hydrolysis. Melibiose, maltose, cellobiose, and turanose were not hydrolyzed by the resin treatment while raffinose and melezitose were partially hydrolyzed. Starch and inulin were treated separately with resin and HCl. No reducing sugars were formed from starch with either the resin or acid hydrolysis while the acid-hydrolyzed inulin titrated completely as reducing sugar, but from the resin treatment only a trace of reducing sugar was detected. The resin method was compared with HCl and invertase methods for the determination of non-reducing sugars in tomato, tobacco, and barley extracts. All 3 methods gave similar results. The recovery of sucrose added to a plant extract was practically quantitative following the resin hydrolysis. ABSTRACT
AUTHORS: G. R. Noggle

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0000497473 BIOSIS NO.: 19441800020468

The effect of carbon dioxide upon the changes in the sugar content of certain vegetables in cold storage

AUTHOR: Denny F E; Thornton Norwood C; Schroeder Eltora M

JOURNAL: CONTR BOYCE THOMPSON INST 13 ((6)): p295-311 1944 1944

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Unspecified

ABSTRACT: Roots of carrot (*Daucus carota* var. *sativa*) and parsnip (*Pastinaca sativa*), green pods of lima bean (*Phaseolus limensis* var. *limenans*), and tubers of Jerusalem artichoke (*Helianthus tuberosus*) were placed in storage at 5[degree] C in large galvanized iron containers in which the following concs, of CO₂ were maintained 0, 2.5, 7.5, and 22.5% by volume. At intervals, samples were removed for sugar analyses of the roots, tubers, and the seeds removed from the green pods of lima bean. CO₂ retarded the increase in reducing sugar which occurred in carrot and Jerusalem artichoke, but it increased the rate in parsnip. No reducing sugar was found in lima bean seeds under any storage condition, or at any stage of storage. CO₂ accelerated the increase in sucrose which occurred in parsnip, but retarded the sucrose increase in Jerusalem artichoke. With carrot and lima bean, sucrose decreased during cold storage and CO₂ retarded this decrease. Parsnip roots were found to contain a substance or substances which was hydrolyzed by HCl in the cold but not by an active invertase soln. This "additional substance" increased in amt. in the control lot during cold storage but this increase was inhibited by CO₂. Hydrolysis of inulin in the Jerusalem artichoke during cold storage was retarded by CO₂. Retention of

good color and condition of the green pods of lima bean during storage at 5[degree] C was favored by the presence of CO₂. ABSTRACT AUTHORS: Auth. summ

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DIALOG(R)File 5:Biosis Previews(R)

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0000123862 BIOSIS NO.: 19280200009509

Researches on the formation of diastase by *Aspergillus niger*. II

AUTHOR: FUNKE G L

JOURNAL: REC TRAV BOT NEERL 23 ((1/2)): p200-244 1926 1926

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Unspecified

ABSTRACT: A culture solution which contains a **reducing** **sugar** together with a neutral phosphate is colored yellow or light brown during sterilizing, due to substances arising from the action of the alkali of the glass upon the sugar; acid phosphate binds the alkali, so that the sugar remains unaltered. These substances retard growth and promote formation of amylase when fructose is used but prevent it with other sugars. It is indifferent for formation of amylase whether K₂HPO₄ or KH₂PO₄ is used. Glucose and starch further formation of amylase; fructose, mannose, lactose, and **inulin** prevent it. Galactose and mannose do not prevent enzyme production, but their metabolic products do. Glycerine does not favor formation of amylase, nor does it prevent it when mixed with another substance which is conducive to it. Not all sorts of sugars are assimilated to the same degree; galactose and lactose appear to be poor sources of C. It is proposed that there must be a certain close relation of structure between the amylase molecule and that of the substance which is furnished as food supply or which is formed during the lifetime of the fungus, if amylase is to be formed. In many cases "races" of fungi are slight modifications only. ABSTRACT AUTHORS: M. J. Sirks

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0000104495 BIOSIS NO.: 19270100004497

Different types of grasses according to their carbohydrate reserves

ORIGINAL LANGUAGE TITLE: Les divers types de Graminees d'apres la nature de leur reserves hydro-carbonees

AUTHOR: COLIN H; CUGNAC A de

JOURNAL: COMPT REND ACAD SCI [PARIS] 182 ((26)): p1637-1639 1926 1926

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Unspecified

ABSTRACT: Blades of grasses under normal conditions contain no carbohydrate except sucrose and the products of its hydrolysis; the grain consists wholly of dry and starchy albumen. According to the carbohydrates in their stems, grasses may be divided into 2 groups: those containing levulosans, and those without levorotatory polysaccharides. To the 2nd

group, besides sugarcane, sorghum, and maize, belong Phragmites, Arundo, Cynodon, and Brachypodium. These grasses contain no soluble carbohydrates except cane sugar and variable quantities of %%%reducing%%% %%%sugar%%% in leaves, rhizomes, and grains. Usually there is no starch in the culm, but it is in sheaths or rhizomes. Grasses having levulosans are more numerous, but the distribution of these substances is nearly identical in all the species. Several levulosans have been isolated from the grasses, graminin from Arrhenatherum and tritacin from Agropyron repens. Whether there are few or several of these substances in the Gramineae, they have an aggregate of properties in common which separates them from %%%inulin%%% of the Compositae, scillin of the Liliaceae, and irisin of Iris pseudacorus. A table gives analyses of 4 levulosan-grasses and 3 sucrose-grasses. ABSTRACT AUTHORS: A. Chase

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Set	Items	Description
S1	9	INULIN AND (REDUCING() SUGAR)
S2	5	INULIN AND TAGATOSE
S3	992	INULIN AND GLUCOSE
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
S5	48	INULIN AND HEMOGLOBIN
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S7	0	S5 AND TAGATOSE
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S9	0	INULIN AND (PEG?(3W)HEMOGLOBIN)
S10	0	S1 AND S4
S11	2	S4 AND S5

? t s2/7/1-5

2/7/1

DIALOG(R)File 5:Biosis Previews(R)

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17018783 BIOSIS NO.: 200200612294

Utilisation of prebiotic carbohydrates by strains of Lactobacillus reuteri

AUTHOR: Kneifel W (Reprint); Bonaparte C (Reprint); Casas I

AUTHOR ADDRESS: Department of Dairy Research and Bacteriology, University of Agricultural Sciences, Vienna, Austria**Austria

JOURNAL: British Journal of Nutrition 88 (Supplement 1): pS111-S112

September 1, 2002 2002

MEDIUM: print

CONFERENCE/MEETING: 2001 Yakult International Conference on Probiotics and Health London, England September 13-14, 2001; 20010913

SPONSOR: The Nutrition Society

ISSN: 0007-1145

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Citation

LANGUAGE: English

2/7/2

DIALOG(R)File 5:Biosis Previews(R)

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16966731 BIOSIS NO.: 200200560242

Carnobacterium viridans sp. nov., an alkaliphilic, facultative anaerobe isolated from refrigerated, vacuum-packed bologna sausage

AUTHOR: Holley Richard A (Reprint); Guan Tat Yee; Peirson Michael; Yost
Christopher K
AUTHOR ADDRESS: Department of Food Science, Faculty of Agricultural and
Food Sciences, University of Manitoba, Winnipeg, Manitoba, R3T 2N2,
Canada**Canada
JOURNAL: International Journal of Systematic and Evolutionary Microbiology
52 (5): p1881-1885 September, 2002 2002
MEDIUM: print
ISSN: 1466-5026
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A facultatively anaerobic, non-spore-forming, psychrophilic,
Gram-positive, non-aciduric but alkaliphilic, rod-shaped bacterium
(MPL-11T) was found to be responsible for green discoloration of
refrigerated vacuum-packaged bologna upon opening of the package.
Although *Aerococcus viridans*, which had been implicated earlier in
causing the same problem, was also found, this is the first report of
discoloration caused by an organism shown to be a species of
Carnobacterium. Bacterial discoloration was caused by H₂O₂ production
upon exposure of the meat to air. Strain MPL-11T is catalase- and
oxidase-negative. It is not motile and does not reduce nitrate to nitrite
or produce ammonia from arginine. It does not grow in acetate-containing
broth or agar (Rogosa) or produce H₂S. The peptidoglycan is of the
meso-diaminopimelic acid type and it produces predominantly L(+)-lactic
acid from glucose. It grows from at least 2 to 30 degreeC over a pH range
from 5.5 to 9.1. Ribotyping suggested that strain MPL-11T could be a
species of either *Lactobacillus* or *Carnobacterium*, but analysis using DNA
sequences from the 16S rRNA gene showed conclusively that the organism
belonged to the genus *Carnobacterium*. Since acid is not produced from
amygdalin, %inulin%, mannitol, methyl alpha-D-glucoside or D-xylose,
the organism differs from the seven described species of *Carnobacterium*.
In addition, strain MPL-11T is the first member of the genus found that
does not produce acid from ribose. It is capable of acid
production/growth on galactose, glucose, fructose, mannose,
N-acetylglucosamine, aesculin, cellobiose, maltose, lactose, sucrose,
trehalose and %tagatose%. Although extremely salt tolerant, it does
not grow in gtoreq 4% NaCl. On the basis of phenotypic and genotypic
data, it is concluded that this isolate represents a separate, novel
species. Accordingly, the name *Carnobacterium viridans* sp. nov. is
proposed. The type strain is strain MPL-11T (= ATCC BAA-336T = DSM
14451T).

2/7/3

DIALOG(R)File 5:Biosis Previews(R)
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14667641 BIOSIS NO.: 199800461888
The forgotten, but important sources of dietary fiber
AUTHOR: Gordon D T (Reprint)
AUTHOR ADDRESS: Dep. Cereal Sci., North Dakota State Univ., Harris Hall
110, Fargo, ND 58105, USA**USA
JOURNAL: Cereal Foods World 43 (7): p515 July, 1998 1998
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the American Association of Cereal

Chemists Minneapolis, Minnesota, USA September 13-17, 1998; 19980913
SPONSOR: American Association of Cereal Chemists
ISSN: 0146-6283
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/4

DIALOG(R)File 5:Biosis Previews(R)
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09204936 BIOSIS NO.: 198886044857
A SIMPLE AND RAPID DETERMINATION OF KETOSES BY CIRCULAR DICHROISM
AUTHOR: KIMURA A (Reprint); CHIBA S; YONEYAMA M
AUTHOR ADDRESS: DEP AGRIC CHEM, FAC AGRIC, HOKKAIDO UNIV, SAPPORO 060**
JAPAN
JOURNAL: Carbohydrate Research 175 (1): p17-24 1988
ISSN: 0008-6215
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A simple and rapid determination of D-fructose is possible by circular dichroism measurement. The proportionality of the ellipticity to the concentration of D-fructose extends up to a concentration of 4.5 M (81%, w/v). A constant value of the ellipticity was observed within 15 minutes after preparation of the solution. Such carbohydrates as aldoses, sucrose, and inulin, and several conventional inorganic salts, do not affect the determination. The ellipticity was found to depend on the temperature of measurement. This assay method was successfully applied to some reactions: i.e., α -D-glucosidase-catalyzed hydrolysis of sucrose, glucose isomerase-catalyzed isomerization, and acid hydrolysis of inulin. The method was also found applicable to such other ketoses as D-tagatose, L-sorbose, and turanose.

2/7/5

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06032606 BIOSIS NO.: 198070064093
CHARACTERS OF LACTOBACILLUS-CORYNIFORMIS ISOLATED FROM AN IRAQI CHEESE
AUTHOR: HEGAZI F Z (Reprint); ABO-ELNAGA I G
AUTHOR ADDRESS: DAIRY DEP, FAC AGRIC, UNIV ASSIUT, ASSIUT, IRAQ**IRAQ
JOURNAL: Zentralblatt fuer Bakteriologie Parasitenkunde
Infektionskrankheiten und Hygiene Zweite Naturwissenschaftliche Abteilung
Mikrobiologie der Landwirtschaft der Technologie und des Umweltschutzes
135 (3): p205-211 1980
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The cultural, physiological and biochemical characters of 21 strains of *L. coryniformis*, isolated from Iraqi cheese, were investigated. Some of the strains grew at 45.degree. C. All possessed the following characteristics: they did not contain DAP [diaminopimelate]; no

growth in 4% taurocholate or in 9% NaCl; growth in 6.5% NaCl; milk mostly coagulated within 3-60 days with final activity of 0.85-1.09% and pH 4.05; xylose, (D+)%tagatose%, %inulin% and trehalose not fermented; ribose fermented by only 1 strain and arabinose by another; pyruvate, malate and fumarate decomposed in the presence of glucose with formation of CO₂; CO₂ was produced from gluconate by 20 out of 21 strains. The mean total amount of lactate, produced after 41 days at 30.degree. C, was 42.6 .+- . 2.5 .mu.mol/ml, of L(+)lactate 17.8 .+- . 1.1, and of % (+)lactate of total lactate 42.3 .+- . 1.7. The isolates degraded pyruvate (111 .mu.mol/ml) in the presence of glucose (55.5 .mu.mol/ml) with lactate as the major product, together with acetate 5.8%, ethanol 8.15%, acetoin 1.95% and diacetyl 2.50% yield on a molar basis after 60 days at 30.degree. C. Diacetyl and acetoin could be formed from pyruvate plus glucose, but not from either glucose alone, citrate alone, or from citrate plus glucose. The mean total amount of diacetyl plus acetoin, after 26 days at 30.degree. C, was 1059.6 .+- . 24.0 .mu.g/ml, of diacetyl 92.8 .+- . 2.2 and of % diacetyl of the total diacetyl plus acetoin was 8.8 .+- . 0.3. *L. coryniformis* differs from *L. plantarum* in morphology, in not containing DAP, in failure to grow in 4% taurocholate, in not fermenting ribose and trehalose, and in not decomposing tartrate.

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Set	Items	Description
S1	9	INULIN AND (REDUCING()) SUGAR)
S2	5	INULIN AND TAGATOSE
S3	992	INULIN AND GLUCOSE
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
S5	48	INULIN AND HEMOGLOBIN
S6	8	S5 AND GLUCOSE
S7	0	S5 AND TAGATOSE
S8	0	INULIN AND (PEG?(3)HEMOGLOBIN)
S9	0	INULIN AND (PEG?(3W)HEMOGLOBIN)
S10	0	S1 AND S4
S11	2	S4 AND S5

? t s6/7/1-8

6/7/1

DIALOG(R)File 5:Biosis Previews(R)

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17747487 BIOSIS NO.: 200400118244

Differential susceptibility to diabetic nephropathy in inbred mouse strains.

AUTHOR: Qi Zhonghua (Reprint); Jin Jianping (Reprint); Fogo Agnes B (Reprint); Breyer Matthew D (Reprint)

AUTHOR ADDRESS: Medicine/Nephrology, Vanderbilt University, Nashville, TN, USA**USA

JOURNAL: Journal of the American Society of Nephrology 14 (Abstracts Issue): p595A November 2003 2003

MEDIUM: print

CONFERENCE/MEETING: Meeting of the American Society of Nephrology Renal Week San Diego, CA, USA November 12-17, 2003; 20031112

SPONSOR: American Society of Nephrology

ISSN: 1046-6673

DOCUMENT TYPE: Meeting; Meeting Poster; Meeting Abstract

RECORD TYPE: Citation

LANGUAGE: English

6/7/2

DIALOG(R)File 5:Biosis Previews(R)

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13745170 BIOSIS NO.: 199799379230

Angiotensin converting enzyme gene polymorphism and renal hemodynamic function in early diabetes

AUTHOR: Miller Judith A (Reprint); Scholey James W; Thai Kerri; Pei York P C

AUTHOR ADDRESS: Women's Coll. Hosp., Suite 424, Burton Hall, 60 Grosvenor St., Toronto, ON M5S 1B6, Canada**Canada

JOURNAL: Kidney International 51 (1): p119-124 1997 1997

ISSN: 0085-2538

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: An insertion/deletion (I/D) of the human angiotensin converting enzyme (ACE) gene is a major determinant of circulating ACE levels. Recent studies suggest that the ACE I/D polymorphism may influence the risk of developing nephropathy in patients with insulin dependent diabetes mellitus (IDDM), although the mechanism responsible for the effect is unknown. Since an early increase in glomerular filtration rate (GFR) may also be a risk factor for the development of diabetic nephropathy, we sought to determine if the ACE I/D polymorphism influenced renal hemodynamic function in patients with IDDM. Genomic DNA was obtained from 39 normotensive male and female patients with uncomplicated IDDM (mean duration 3.4 years; range 1 to 6 years), and from 20 non diabetic control subjects. The ACE I/D polymorphism was determined using the polymerase chain reaction. Subjects were divided into three groups based on their ACE genotype. Values for GFR, renal plasma flow (ERPF), filtration fraction, and renal vascular resistance were determined in both groups using classic inulin and paraaminohippurate clearance techniques. Blood glucose was maintained between 4 to 6 mmol/liter in the patients with IDDM using a modified euglycemic clamp technique. Mean values for GFR were significantly greater in patients homozygous for the I allele (143 ± 7 ml/min/1.73 m⁻²) compared to patients homozygous for the D allele (121 ± 3 ml/min/1.73 m⁻², $p < 0.01$), while the mean GFR values for the heterozygous patients were intermediate. ERPF was also significantly greater in patients homozygous for the I allele (850 ± 10 ml/min/1.73 m⁻²) compared to patients homozygous for the D allele (672 ± 31 ml/min/1.73 m⁻², $P < 0.04$), while there were no differences in the values for mean arterial pressure, glycosylated hemoglobin , or albumin excretion rates amongst the groups. There was no dominant effect of the ACE gene I/D polymorphism in the control group. These results suggest that: (1) the ACE gene I/D polymorphism influences glomerular filtration and renal plasma flow rates in patients with early uncomplicated IDDM; and (2) differences in renal hemodynamic function do not appear to explain the protection against the development of diabetic nephropathy offered by the I allele.

6/7/3

DIALOG(R)File 5:Biosis Previews(R)

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10857265 BIOSIS NO.: 199192103036

POTENTIAL DELETERIOUS IMPACT OF DIETARY SALT RESTRICTION ON CARDIOVASCULAR RISK FACTORS

AUTHOR: WEDER A B (Reprint); EGAN B M

AUTHOR ADDRESS: UNIV MICH MED CENT, DIVISION HYPERTENSION, 3918 TAUBMAN CENT, ANN ARBOR, MICH 48109-0356, USA**USA

JOURNAL: Klinische Wochenschrift 69 (SUPPL. 25): p45-50 1991

ISSN: 0023-2173

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Excessive intake of dietary salt is thought to promote hypertension Western societies, and some have recommended salt restriction for the general population. While such restriction is thought to be innocuous, few studies have examined the impact of dietary salt on cardiovascular risk factors other than blood pressure. In a randomized, placebo-controlled, double blinded comparison of one week periods of 20 vs. 208 mEq/d NaCl intake in 27 hypertensives and normotensives, we found that salt restriction had no significant effect on blood pressure ($p = 0.45$) and a generally adverse impact on risk factors for cardiovascular disease. Stringent, short-term dietary salt restriction caused increases in total and low-density lipoprotein cholesterol that were of borderline significance ($p = 0.07$). These lipid effects probably resulted from plasma volume contraction, as they were coincident with significant rises in %hemoglobin% ($p = 0.01$), hematocrit ($p < 0.001$), total protein ($p < 0.01$) and albumin ($p = 0.01$); such changes may act together to increase whole-blood viscosity. In addition, plasma norepinephrine ($p = 0.02$), fasting plasma insulin ($p = 0.02$) and %glucose%-to-insulin ratio ($p = 0.01$) increased during salt restriction. The potentially adverse impact of dietary salt restriction on the risk factor profile for cardiovascular disease suggests that further studies are necessary before a reduction in dietary salt intake can be prescribed for the general population.

6/7/4

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09812878 BIOSIS NO.: 198988127993

ENDOTHELIAL RECEPTOR-MEDIATED BINDING OF %GLUCOSE%-MODIFIED ALBUMIN IS ASSOCIATED WITH INCREASED MONOLAYER PERMEABILITY AND MODULATION OF CELL SURFACE COAGULANT PROPERTIES

AUTHOR: ESPOSITIO C (Reprint); GERLACH H; BRETT J; STERN D; VLASSARA H

AUTHOR ADDRESS: LAB MED BIOCHEM, ROCKEFELLER UNIV, 1230 YORK AVE, BOX 277, NEW YORK 10021, USA**USA

JOURNAL: Journal of Experimental Medicine 170 (4): p1387-1408 1989

ISSN: 0022-1007

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Advanced glycosylation end products (AGE) of proteins accumulate in the vasculature with diabetes and aging, and are thought to be associated with vascular complications. This led us to examine the

interaction of AGE-BSA as a prototype of this class of nonenzymatically glycosylated proteins subjected to further processing, with endothelium. Incubation of 125I-AGE-BSA with cultured bovine endothelium resulted in time-dependent, saturable binding that was half-maximal at a concentration of .apprx.100 nM. Although unlabeled normal BSA was not a competitor, unlabeled AGE-BSA was an effective competitor of 125I-AGE-BSA-endothelial cell interaction. In addition, AGE modification of two alternative proteins, %hemoglobin% and ribonuclease, rendered them inhibitors of 125I-AGE-BSA binding to endothelium, although the native, unmodified forms of these proteins were not. At 37.degree.C, binding of 125I-AGE-BSA or gold-labeled AGE-BSA was followed by internalization and subsequent segregation either to a lysosomal compartment or to the endothelial-derived matrix after transcytosis. Exposure of endothelium to AGE-BSA led to perturbation of two important endothelial cell homeostatic properties, coagulant and barrier function. AGE-BSA downregulated the anticoagulant endothelial cofactor thrombomodulin, and induced synthesis and cell surface expression of the procoagulant cofactor tissue factor over the same range of concentrations that resulted in occupancy of cell surface AGE-BSA binding sites. In addition, AGE-BSA increased endothelial permeability, resulting in accelerated passage of an inert macromolecular tracer, [3H]%%inulin%%, across the monolayer. These results indicate that AGE derivatives of proteins, potentially important constituents of pathologic vascular tissue, bind to specific sites on the endothelial cell surface and modulate central endothelial cell functions. The interaction of AGE-modified proteins with endothelium may play an important role in the early stages of increased vascular permeability, as well as vessel wall-related abnormalities of the coagulation system, characteristic of diabetes and aging.

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08572060 BIOSIS NO.: 198783050951

STUDY OF NONELECTROLYTE TRANSPORT THROUGH CELLULOSE HYDRATE MEMBRANES

AUTHOR: BROMBERG L E (Reprint); RUDMAN A R; VENGEROVA N A; EL'TSEFON B S

AUTHOR ADDRESS: ALL-UNION RES INST MED POLYM, MOSCOW, USSR**USSR

JOURNAL: Khimiko-Farmatsevticheskii Zhurnal 20 (6): p747-753 1986

ISSN: 0023-1134

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: RUSSIAN

ABSTRACT: The permeability was studied of water-soluble nonelectrolytes through the cuprophane and diacell membranes used for hemodialysis. The following substances were considered: tritiated water, urea, creatinine, %%glucose%%, %%glucose%%-6-phosphate, sucrose, raffinose, vitamin B12, %%inulin%%, RNase, cytochrome C, lysozyme, myoglobin, trypsin, ovalbumin and human %hemoglobin%. The parameters of the transport were established based on the concepts of free volume and thermodynamic assumptions. Diffusion and hydraulic permeability of the membranes were determined, as were correlations between the reflection coefficients and molecular weights of the nonelectrolytes.

6/7/6

DIALOG(R)File 5:Biosis Previews(R)

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07693292 BIOSIS NO.: 198580002187

PERMSELECTIVITY OF THE PERITONEAL MEMBRANE

AUTHOR: RIPPE B (Reprint); PERRY M A; GRANGER D N

AUTHOR ADDRESS: SECTION NEPHROLOGY, MED CLINIC I, SAHLGRENSKA HOSP S-413,
45 GOTEBOG, SWEDEN**SWEDEN

JOURNAL: Microvascular Research 29 (1): p89-102 1985

ISSN: 0026-2862

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: To investigate the osmotic barrier characteristics of the peritoneal membrane during conditions similar to peritoneal dialysis in man, net transperitoneal fluid movement was measured in 20 cats following intraabdominal placement of isotonic saline and hypertonic solutions of NaCl, %glucose%, raffinose and %inulin%. Isooncotic solutions of Hb and albumin and 2 sulfated high-MW dextrans were investigated. Transperitoneal fluid movement was measured by a volume recovery method. Oncotic pressures of test solutions and plasma were measured by osmometry. Peritoneal osmotic conductances were calculated from the rate of transperitoneal water movement and the difference in osmotic pressures between the test solution and isotonic saline. The average %glucose% osmotic conductance/U body surface area was found to be 2.3 ± 0.18 .times. 10^{-3} ml .cntdot. min⁻¹ mm Hg⁻¹ .cntdot. m⁻² in good agreement with previous reports, and the %glucose% osmotic reflection coefficient (.omega.) was estimated to be 0.02. All the osmotic conductances measured could be fitted to a peritoneal equivalent pore radius .apprx. 6 nm according to current hydrodynamic theories. The peritoneal membrane filtration coefficient was estimated to be 0.12 ml .cntdot. min⁻¹ .cntdot. mm Hg⁻¹ .cntdot. m⁻², of which 0.5-1% was found to be due to transcellular water flow. The peritoneum apparently is a highly selective membrane with restrictive properties comparable to those reported for continuous capillary beds.

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0000924479 BIOSIS NO.: 19583200012041

The general form of excretion in the lobster, Homarus

AUTHOR: BURGER J WENDELL

AUTHOR ADDRESS: Trinity Coll., Hartford, Conn.

JOURNAL: BIOL BULL 113 ((2)): p207-223 1957 1957

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Unspecified

ABSTRACT: A method was found for the repeated evacuation of nephridial bladders. Experimental analysis showed the nephridia capable of concentrating phenol red, para-aminohippurate (PAH), Mg, and sulfate. At normal blood levels %glucose% and inorganic phosphate are excluded from the urine. The secretory or exclusion powers of the nephridia are

swamped by artificially high blood levels. Nephridia are indifferent to %inulin%, bromsulfalein, elasmobranch %hemoglobin% and plasma protein, sodium, and chloride. Exogenous urea is lost through the gills. The gills are relatively impermeable to magnesium, sulphate, phenol red but permeable to water and NaCl. A variety of chemical measurements for sea water, blood, and urine were made. In sea water, the distribution of NaCl between sea water, blood, and urine seems to be passive. In dilute sea water, blood chloride is elevated, presumably by some active process in the gills. Urinary chloride is lost at the elevated blood levels indicating a lack of nephridial ability to actively handle chloride. Living lobsters may be completely anuric, but the so-called normal urine flow is about 1 ml/hour/0.5 kg. Anuric lobsters can be converted into normal urinators by the transfusion of blood. While water and NaCl enter largely through the gills, multi-valent ions and organic molecules enter through the gut. The lobster drinks sea water with its food and also intermittently on an empty stomach. This water is absorbed. The volume of the sea water drunk is however, insufficient to account for the volume of urine. For various substances, the nephridia and the gills have individual properties. The nephridia depend on high urine flows rather than on high secretion. ABSTRACT AUTHORS: J. W. Burger

6/7/8

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0000690502 BIOSIS NO.: 19512500023478

Normal %hemoglobin% clearances in chronic proteinuria

AUTHOR: BRANDT J LEONARD; FRANK ROBERT; LICHTMAN HERBERT C

AUTHOR ADDRESS: State U. New York, Brooklyn

JOURNAL: PROC SOC EXPTL BIOL AND MED 74 ((4)): p863-865 1950 1950

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Unspecified

ABSTRACT: The ratio of simultaneously detd. renal clearance of Hb and %inulin%, in successive clearance periods in a group of 5 control and 3 patients with the nephrotic syndrome, indicates that the overall glomerular porosity, for Hb, of the patients with proteinuria is no greater than that of normals. All patients were tested 10 hrs. after their last meal and hydrated with 500-1000 cc. of water prior to the test. Continuous infusion was used throughout with a priming infusion of 75-100 cc. of 6% Hb soln. and 30 cc. of 10% %inulin%; sustaining infusion, 4 cc./min., contained 3% Hb and a proper amt. of %inulin% using 5% %glucose%. ABSTRACT AUTHORS: A. E. Schaefer

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Set	Items	Description
S1	9	INULIN AND (REDUCING() SUGAR)
S2	5	INULIN AND TAGATOSE
S3	992	INULIN AND GLUCOSE
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
S5	48	INULIN AND HEMOGLOBIN
S6	8	S5 AND GLUCOSE
S7	0	S5 AND TAGATOSE
S8	0	INULIN AND (PEG?(3) HEMOGLOBIN)
S9	0	INULIN AND (PEG?(3W) HEMOGLOBIN)

S10 0 S1 AND S4
 S11 2 S4 AND S5
 ? s inulin and (purif? and protein)
 9586 INULIN
 393958 PURIF?
 1957135 PROTEIN
 S12 88 INULIN AND (PURIF? AND PROTEIN)
 ? s s3 and (purif? and protein)
 992 S3
 393958 PURIF?
 1957135 PROTEIN
 S13 12 S3 AND (PURIF? AND PROTEIN)
 ? t s13/7/1-12

13/7/1

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0020892799 BIOSIS NO.: 200900233133

Production of beta-fructofuranosidases by *Aspergillus niveus* using
 agroindustrial residues as carbon sources: Characterization of an
 intracellular enzyme accumulated in the presence of %glucose%%
 AUTHOR: Guimaraes Luis Henrique S (Reprint); Somera Alexandre Favarin;
 Terenzi Hector Francisco; Teixeira de Moraes Polizeli Maria de Lourdes;
 Jorge Joao Atilio

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JOURNAL: Process Biochemistry 44 (2): p237-241 FEB 2009 2009

ITEM IDENTIFIER: doi:10.1016/j.procbio.2008.10.011

ISSN: 1359-5113

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The production of beta-fructofuranosidases by *Aspergillus niveus*,
 cultivated under submerged fermentation using agroindustrial residues,
 was investigated. The highest productivity of beta-fructofuranosidases
 was obtained in Khanna medium supplemented with sugar cane bagasse as
 carbon source. %Glucose%% enhanced the production of the intracellular
 enzyme, whereas that of the extracellular one was decreased. The
 intracellular beta-fructofuranosidase was a trimeric %protein%% of
 approximately 141 kDa (gel filtration) with 53.5% carbohydrate content,
 composed of 57 kDa monomers (SDS-PAGE). The optimum temperature and
 optimum pH were 60 degrees C and 4.5, respectively. The %purified%%
 enzyme showed good thermal stability and exhibited a half-life of 53 min
 at 60 degrees C. beta-Fructofuranosidase activity was slightly activated
 by Cu²⁺, Mn²⁺, Mg²⁺, and Na⁺ at 1 mM concentration. The enzyme hydrolyzed
 sucrose, raffinose, and %inulin%%, with K-d values of 5.78 mM, 5.74
 mM, and 1.74 mM, respectively. (C) 2008 Elsevier Ltd. All rights
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13/7/2

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0019974616 BIOSIS NO.: 200800021555

Production and characterization of a thermostable extracellular
beta-D-fructofuranosidase produced by *Aspergillus ochraceus* with
agroindustrial residues as carbon sources

AUTHOR: Guimaraes Luis Henrique S (Reprint); Terenzi Hector Francisco;

Polizeli Maria De Lourdes Teixeira De Moraes; Jorge Joao Atilio

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Ribeirao Preto, Brazil**Brazil

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JOURNAL: Enzyme and Microbial Technology 42 (1): p52-57 DEC 3 2007 2007

ITEM IDENTIFIER: doi:10.1016/j.enzniictec.2007.07.021

ISSN: 0141-0229

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The filamentous fungus *Aspergillus ochraceus* produced high levels of a thermostable extracellular P-D-fructofuranosidase (EC 3.2.1.26) when cultured for 96 h, at 40 degrees C, in Khanna medium supplemented with sugar cane bagasse as carbon source. The enzyme was 7.1-fold purified, with a recovery of 24%, by two chromatographic steps in DEAE-cellulose and Sephacryl S-200. The purified enzyme was homogeneous according to electrophoretic criteria. P-D-Fructofuranosidase was a homodimeric glycoprotein with 41% carbohydrate content and apparent molecular mass of 135 kDa, estimated by gel filtration in Sephacryl S-200, or 79 kDa by SDS-PAGE. Optima of pH and temperature were 4.5 and 60 degrees C, respectively. The enzyme showed a t(50) of 60 min at 60 degrees C. The enzyme activity was stimulated by Mn2+ (57%), Mg2+ (50%), Na+ (35%) and Ba2+ (20%), and inhibited by Cu2+ and Hg2+. Glucose at 40 mM stimulated the *A. ochraceus* extracellular beta-fructofuranosidase in about 2.68-fold. The enzyme hydrolyzed raffinose, sucrose and inulin, exhibiting K-m of 7.37, 13.4 and 2.66 mM, and V-max., of 22.39, 42.13 and 3.14 U mg(-1) protein, respectively. Transfructosylation reactions were not detected, since glucose and fructose were the only products from sucrose hydrolysis. (C) 2007 Elsevier Inc. All rights reserved.

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0019717242 BIOSIS NO.: 200700376983

Molecular and biochemical characterization of a novel intracellular
invertase from *Aspergillus niger* with transfructosylating activity

AUTHOR: Goosen Coenie; Yuan Xiao-Lian; van Munster Jolanda M; Ram Arthur F
J; van der Maarel Marc J E C (Reprint); Dijkhuizen Lubbert

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JOURNAL: Eukaryotic Cell 6 (4): p674-681 APR 2007 2007

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ISSN: 1535-9778

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RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A novel subfamily of putative intracellular invertase enzymes (glycoside hydrolase family 32) has previously been identified in fungal genomes. Here, we report phylogenetic, molecular, and biochemical characteristics of SucB, one of two novel intracellular invertases identified in *Aspergillus niger*. The *sucB* gene was expressed in *Escherichia coli* and an invertase-negative strain of *Saccharomyces cerevisiae*. Enzyme **purified** from *E. coli* lysate displayed a molecular mass of 75 kDa, judging from sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. Its optimum pH and temperature for sucrose hydrolysis were determined to be 5.0 and 37 to 40 degrees C, respectively. In addition to sucrose, the enzyme hydrolyzed I-kestose, nystose, and raffinose but not **inulin** and levan. SucB produced 1-kestose and nystose from sucrose and 1-kestose, respectively. With nystose as a substrate, products up to a degree of polymerization of 4 were observed. SucB displayed typical Michaelis-Menten kinetics with substrate inhibition on sucrose (apparent K_m , K_i , and V_{max} of 2.0 +/- 0.2 mM, 268.1 +/- 18.1 mM, and 6.6 +/- 0.2 $\mu\text{mol min}^{-1}\text{mg}^{-1}$ of **protein** [total activity], respectively). At sucrose concentrations up to 400 mM, transfructosylation (FTF) activity contributed approximately 20 to 30% to total activity. At higher sucrose concentrations, FTF activity increased to up to 50% of total activity. Disruption of *sucB* in *A. niger* resulted in an earlier onset of sporulation on solid medium containing various carbon sources, whereas no alteration of growth in liquid culture medium was observed. SucB thus does not play an essential role in **inulin** or sucrose catabolism in *A. niger* but may be needed for the intracellular conversion of sucrose to fructose, **glucose**, and small oligosaccharides.

13/7/4

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19080483 BIOSIS NO.: 200600425878

Isolation and characterization of a novel lectin from the mushroom

Armillaria luteo-virens

AUTHOR: Feng K; Liu Q H; Ng T B; Liu H Z; Li J Q; Chen G; Sheng H Y; Xie Z L; Wang H X (Reprint)

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JOURNAL: Biochemical and Biophysical Research Communications 345 (4): p

1573-1578 JUL 14 2006 2006

ISSN: 0006-291X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: From the dried fruiting bodies of the Mushroom *Armillaria luteo-virens*, a dimeric lectin with a molecular mass of 29.4 kDa has been isolated. The **purification** procedure involved $(\text{NH}_4)_2\text{SO}_4$ precipitation. ion exchange chromatography oil DEAE-cellulose, CM-cellulose, and Q-Sepharose, and gel filtration by fast **protein** liquid chromatography on Superdex 75. The hemagglutinating activity of the lectin could not be inhibited by simple sugars but was inhibited by

the polysaccharide *inulin*. The activity was stable up to 70 degrees C but was acid and alkali-labile. Salts including FeCl₃, AlCl₃, and ZnCl₂ inhibited the activity whereas MgCl₂, MnCl₂, and CaCl₂ did not. The lectin stimulated mitogenic response Of mouse splenocytes with the maximal response achieved by 1 μ M lectin. Proliferation of tumor cells including MBL2 cells, HeLa cells, and L1210 cells was inhibited by the lectin with an IC₅₀ of 2.5, 5, and 10 μ M, respectively. However, proliferation of HepG2 cells was not affected. The novel aspects of the isolated lectin include a novel N-terminal sequence, fair thermostability, acid stability, and alkali stability, together with potent mitogenic activity toward spleen cells and antiproliferative activity toward tumor cells. (c) 2006 Elsevier Inc. All rights reserved.

13/7/5

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18086156 BIOSIS NO.: 200400467385

Purification and characterization of inulinase from *Aspergillus niger* AF10 expressed in *Pichia pastoris*

AUTHOR: Zhang Linghua ' (Reprint); Zhao Changxin; Zhu Daochen; Ohta Yoshiyuki; Wang Yunji

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JOURNAL: Protein Expression and Purification 35 (2): p272-275 June 2004 2004

MEDIUM: print

ISSN: 1046-5928

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The *inuA1* gene encoding an exoinulinase from *Aspergillus niger* AF10 was expressed in *Pichia pastoris*, and the recombinant enzyme activity was 316 U/ml in a 5 L fermentor, with the inulinase *protein* accounting for 35% of the total *protein* of fermentation broth. The hydrolysis rate of *mulin* can reach 92%, with a 25 U/g *inulin* enzyme addition, and 90% of fructose content after 6 h. *Glucose* can significantly inhibit the enzymatic hydrolysis of *inulin*. This is the first report of *glucose* inhibition of inulinase-catalyzed hydrolysis. Copyright 2004 Elsevier Inc. All rights reserved.

13/7/6

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15772036 BIOSIS NO.: 200000490349

Production, *purification* and characterization of an extracellular inulinase from *Kluyveromyces marxianus* var. *bulgaricus*

AUTHOR: Kushi R T; Monti R; Contiero J (Reprint)

AUTHOR ADDRESS: Laboratorio de Bioquimica Industrial, Instituto de Quimica de Araraquara-UNESP, Rua Prof. Francisco Degni S/N, Araraquara, SP, 14801-970, Brazil**Brazil

JOURNAL: Journal of Industrial Microbiology and Biotechnology 25 (2): p
63-69 August, 2000 2000
MEDIUM: print
ISSN: 1367-5435
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The yeast *Kluyveromyces marxianus* var. *bulgaricus* produced large amounts of extracellular inulinase activity when grown on inulin, sucrose, fructose and glucose as carbon source. This protein has been purified to homogeneity by using successive DEAE-Trisacryl Plus and Superose 6HR 10/30 columns. The purified enzyme showed a relative molecular weight of 57 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 77 kDa by gel filtration in Superose 6 HR 10/30. Analysis by SDS-PAGE showed a unique polypeptide band with Coomassie Blue stain and nondenaturing PAGE of the purified enzyme obtained from media with different carbon sources showed the band, too, when stained for glucose oxidase activity. The optimal hydrolysis temperature for sucrose, raffinose and inulin was 55°C and the optimal pH for sucrose was 4.75. The apparent Km values for sucrose, raffinose and inulin are 4.58, 7.41 and 86.9 mg/ml, respectively. Thin layer chromatography showed that inulinase from *K. marxianus* var. *bulgaricus* was capable of hydrolyzing different substrates (sucrose, raffinose and inulin), releasing monosaccharides and oligosaccharides. The results obtained suggest the hypothesis that enzyme production was constitutive.

13/7/7

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15107857 BIOSIS NO.: 199900367517
Production and characterization of raffinose-hydrolysing and invertase activities of *Aspergillus fumigatus*
AUTHOR: de Rezende S T (Reprint); Felix C R
AUTHOR ADDRESS: Departamento de Bioquímica e Biologia Molecular,
Universidade Federal de Vicosa, 36.571-000, Vicosa, MG, Brazil**Brazil
JOURNAL: Folia Microbiologica 44 (2): p191-195 1999 1999
MEDIUM: print
ISSN: 0015-5632
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Raffinose-type galactose oligosaccharides constitute a substantial part (40 %) of the soluble sugars present in soybean seeds and are responsible for flatulence following ingestion of soybean and other legumes. Enzymic hydrolysis of these oligosaccharides would improve the nutritional value of soybean milk. *Aspergillus fumigatus* produces substantial raffinose-hydrolysing and invertase activities when grown on wheat straw. Three proteins displaying maximal activity at pH 4.5-5.5 and 55-60 °C and having molar mass of 66.8, 50.3 and 30.2 kDa were purified. Raffinose and sucrose were hydrolyzed with equivalent affinities by each protein. Nevertheless, the Km and Vlim values determined for hydrolysis of sucrose by the 66.8 kDa enzyme differed from

those determined with the 50.3 kDa protein. Glucose was produced when sucrose was the substrate. The three proteins hydrolyzed also stachyose but not melibiose, maltose, inulin or 4-nitrophenyl alpha-D-galactopyranoside. *A. fumigatus* enzymes may be candidates for processing of soybean milk to reduce its flatulence potential.

13/7/8

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14391894 BIOSIS NO.: 199800186141

Purification and properties of inulinase from *Kluyveromyces* sp. Y-85

AUTHOR: Wei Wenling; Yu Xiawen; Dai Ya; Zheng Jing; Xie Zhong

AUTHOR ADDRESS: Dep. Biol., Xiamen Univ., Xiamen 361005, China**China

JOURNAL: Weishengwu Xuebao 37 (6): p443-448 Dec., 1997 1997

MEDIUM: print

ISSN: 0001-6209

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Chinese

ABSTRACT: The crude endocellular inulinase from *Kluyveromyces* sp. Y-85 was purified to two components, designated as EI and EII, using PEG6000-phosphate buffer extraction, (NH₄)₂SO₄ fractionation, DEAE chromatography and gel filtration (Protein-PAK); The crude exocellular inulinase from this strain was purified to Eexo by means of PEG6000-phosphate buffer extraction, double DEAE-Sephac chromatography, Sephadex G-150 gel filtration. EI, EII and Eexo were demonstrated to be homogeneous by Waters 650E protein purification system. Their molecular weights are 42kD, 65kD and 57kD, respectively. All the inulinases were glycoproteins containing a saccharide (from 25% to 35%) and belonged to the endo-inulinase. In addition, EI, EII, Eexo were optimally reactive at pH 4.6, 4.5, 4.6 and at 52 degree C, 52 degree C, 55 degree C, respectively. Ag⁺, Hg²⁺ and PCMB inhibited these enzymes' activity strongly. The products of raw inulin extracted from *Helianthus tuberosus* hydrolyzed by these three enzymes were fructose (86.5%) and glucose (13.5%).

13/7/9

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13037659 BIOSIS NO.: 199598505492

Purification and characterization of the invertase from *Pycnoporus sanguineus*

AUTHOR: Quiroga Emma Nelly; Vattuone Marta Amelia; Sampietro Antonio Rodolfo

AUTHOR ADDRESS: Catedra Fitoquim., Inst. Estudios Vegetales, Fac. Bioquim., Quim. Farm., Univ. Nacl. Tucuman, Ayacucho 461, 4000-San Miguel Tucuman, Argentina**Argentina

JOURNAL: Biochimica et Biophysica Acta 1251 (2): p75-80 1995 1995

ISSN: 0006-3002

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A constitutive invertase (EC 3.2.1.26) was isolated and
%%purified%% by the first time from *Pycnopus sanguineus*. The enzyme
is a glycoprotein. Its relative molecular mass is about 84 000 and its
structure is dimeric, with two identical subunits (about 41 000). The
enzyme is able to attack sucrose, raffinose, stachyose, %%inulin%% and
levan, being sucrose the preferred substrate (K_m 4.89 \pm 0.13 mM).
Fructose was a classical competitive inhibitor, but %%glucose%% was not
an inhibitor of the enzyme. Lectins with specificity toward %%glucose%%
are inhibitors of the enzyme. %%Glucose%% was present in invertase acid
hydrolysates. Unlike higher plant invertases, bovine serum albumin is not
an effector of the *Pycnopus sanguineus* enzyme, and the inhibition by
fructose is not suppressed by this %%protein%%. The properties of the
Pycnopus sanguineus enzyme are discussed and reference to higher plant
invertases.

13/7/10

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12999013 BIOSIS NO.: 199598466846

Continuous production of fructose syrups from %%inulin%% by immobilized
inulinase from *Aspergillus niger* mutant 817

AUTHOR: Nakamura Toyohiko; Ogata Yasuko; Shitara Akichika; Nakamura Akihiro
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JOURNAL: Journal of Fermentation and Bioengineering 80 (2): p164-169 1995
1995

ISSN: 0922-338X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: *Aspergillus niger* mutant 817 was grown in submerged culture with
sucrose. Inulinase was partially %%purified%% from the culture filtrate
by DEAE-Cellulofine A-500 chromatography. The complex enzyme preparation
containing both exo- and endoinulinases was immobilized covalently onto a
porous cellulose derivative, Amino-Cellulofine, by the carbodiimide
method at pH 5.0. The immobilized enzyme had 160 U inulinase activity/g
(wet wt.) of the support, with the immobilization yield of 96% on a
%%protein%% basis and the activity yield of 15%. The maximum inulinase
activity occurred at pH 5.2 and 50 degree C. The immobilized enzyme was
stable in the pH ranges of 4.5 to 6.5 at 30 degree C and 5.0 to 6.0 at 50
degree C. Enzyme stability was retained up to 60 degree C. In a
packed-bed column reactor containing 8 ml of the immobilized inulinase, a
5.0% (w/v) solution (pH 5.0) of pure dahlia %%inulin%% was completely
hydrolyzed at a flow rate of 1.0 ml/min at 40 degree C over a 45-d period
of continuous operation. The volumetric productivity in the reactor was
410 g reducing sugars/l/h. The reaction product was a mixture of 97%
D-fructose and 3% D-%%glucose%%.

13/7/11

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12725675 BIOSIS NO.: 199598193508

Purification and properties of a neutral invertase from the roots of
Cichorium intybus

AUTHOR: Van Den Ende Wim (Reprint); Van Laere Andre

AUTHOR ADDRESS: Dep. Biol., Bot. Inst., K.U. Leuven, Kardinaal Mercierlaan
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JOURNAL: Physiologia Plantarum 93 (2): p241-248 1995 1995

ISSN: 0031-9317

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Multiple activity peaks of neutral invertase (EC 3.2.1.26) were found in chicory roots (Cichorium intybus L. var. foliosum cv. Flash). The main activity peak was ***purified*** by a combination of anion-exchange chromatography hydrophobic interaction chromatography, chromatofocusing and gel filtration. This protocol produced a 77-fold ***purification*** and a specific activity of 1.6 μ -mol (mg ***protein***)-1 min-1. The mass of the enzyme was 260 kDa as estimated by gel filtration and 65 kDa on SDS-PAGE. Optimal activity was found between pH 7 and 7.5. The ***purified*** enzyme exhibited hyperbolic saturation kinetics with a K-m between 10 and 20 mM for sucrose. No other products than ***glucose*** and fructose could be detected. Raffinose was hydrolyzed at a rate of 2.4% relative to sucrose whereas the enzyme did not hydrolyze maltose, cellobiose, trehalose, 1-kestose, 1.1-nystose or ***inulin***. Neutral invertase activity was completely inhibited by HgCl-2 and AgNO-3 and partially inhibited by CoCl-2 and ZnSO-4 (1 mM). Pyridoxal phosphate (K-i apprxeq 500 μ -M), Tris (K-i apprxeq 1.2 mM), ***glucose*** and fructose (K-i apprxeq 16 mM) were strong inhibitors of the enzyme. Fructose and Tris behaved as competitive inhibitors. A possible role for the enzyme's activity in vivo is discussed.

13/7/12

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06971262 BIOSIS NO.: 198376062697

ASSOCIATION OF LIPOSOMES WITH THE ISOLATED PERFUSED RABBIT HEART

AUTHOR: KAYAWAKE S (Reprint); KAKO K J

AUTHOR ADDRESS: DEP PHYSIOL, HEALTH SCI CENTER, UNIV OTTAWA, OTTAWA, ONT
K1N 9A9, CAN**CANADA

JOURNAL: Basic Research in Cardiology 77 (6): p668-681 1982

ISSN: 0300-8428

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Liposomes were prepared from a mixture of either phosphatidylcholine (PC) and cholesterol (Ch) (7:2) (neutral), PC, dicetylphosphate and Ch (4:1:3) (negative), or PC, stearylamine and Ch (4:1:3) (positive). As the lipid phaseliposomal marker, 3H- or 14C-Ch was added. Alternatively, 14C-labeled mannitol, ***glucose*** or ***inulin*** was used as the aqueous phase-marker in some experiments. The liposomes were ***purified*** by Sephadex gel chromatography and by using microfilter. After 4 h of sonication, 95% of the total liposomes were

found to be smaller than 1.2 μm . The entrapment volume was calculated to be 0.9–1.2 $\mu\text{l}/\mu\text{mol}$ of lipid. The ratio of lipid radioactivity and aqueous phase-radioactivity, which were found in a nonfiltrable portion of the perfusate, remained constant during a heart-perfusion period of 30 min, indicating that the liposomes were stable during the experimental period. The wash-out experiment indicated that the liposomes were distributed in a space of the perfused heart nearly as large as the mannitol space. The liposomes were rapidly taken up by the heart during perfusion in a Langendorff manner. The positive liposomes were taken up by the perfused nonischemic heart at a greater rate than were the negative liposomes. The results with perfused ischemic hearts were equivocal. The liposomal label was distributed unequally in the subcellular fractions, namely, relatively greater amounts (per mg $\% \text{protein} \%$) of liposome-bound radioactivity of Ch or mannitol were found in the microsomal fraction than in the mitochondrial or cytosolic fractions of the perfused heart. This distribution pattern was not influenced by the electrical charge of liposomes or by the oxygenation state of the heart perfusion. The accumulation of the liposomal label in the microsomal fraction found in the heart perfusion experiment could not be observed in the experiment in which tissue slices were incubated in the presence of liposomes or in the experiment in which free mannitol was administered in the heart perfusion.

? ds

Set	Items	Description
S1	9	INULIN AND (REDUCING()) SUGAR)
S2	5	INULIN AND TAGATOSE
S3	992	INULIN AND GLUCOSE
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
S5	48	INULIN AND HEMOGLOBIN
S6	8	S5 AND GLUCOSE
S7	0	S5 AND TAGATOSE
S8	0	INULIN AND (PEG?(3)HEMOGLOBIN)
S9	0	INULIN AND (PEG?(3W)HEMOGLOBIN)
S10	0	S1 AND S4
S11	2	S4 AND S5
S12	88	INULIN AND (PURIF? AND PROTEIN)
S13	12	S3 AND (PURIF? AND PROTEIN)

? t s12/7/60-70

12/7/60

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12725675 BIOSIS NO.: 199598193508

$\% \text{Purification} \%$ and properties of a neutral invertase from the roots of *Cichorium intybus*

AUTHOR: Van Den Ende Wim (Reprint); Van Laere Andre

AUTHOR ADDRESS: Dep. Biol., Bot. Inst., K.U. Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium**Belgium

JOURNAL: Physiologia Plantarum 93 (2): p241-248 1995 1995

ISSN: 0031-9317

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Multiple activity peaks of neutral invertase (EC 3.2.1.26) were

found in chicory roots (*Cichorium intybus* L. var. *foliosum* cv. Flash). The main activity peak was **purified** by a combination of anion-exchange chromatography hydrophobic interaction chromatography, chromatofocusing and gel filtration. This protocol produced a 77-fold **purification** and a specific activity of 1.6 $\mu\text{mol (mg protein)}^{-1} \text{ min}^{-1}$. The mass of the enzyme was 260 kDa as estimated by gel filtration and 65 kDa on SDS-PAGE. Optimal activity was found between pH 7 and 7.5. The **purified** enzyme exhibited hyperbolic saturation kinetics with a K_m between 10 and 20 mM for sucrose. No other products than glucose and fructose could be detected. Raffinose was hydrolyzed at a rate of 2.4% relative to sucrose whereas the enzyme did not hydrolyze maltose, cellobiose, trehalose, 1-kestose, 1.1-nystose or **inulin**. Neutral invertase activity was completely inhibited by HgCl_2 and AgNO_3 and partially inhibited by CoCl_2 and ZnSO_4 (1 mM). Pyridoxal phosphate ($K_i \approx 500 \mu\text{M}$), Tris ($K_i \approx 1.2 \text{ mM}$), glucose and fructose ($K_i \approx 16 \text{ mM}$) were strong inhibitors of the enzyme. Fructose and Tris behaved as competitive inhibitors. A possible role for the enzyme's activity in vivo is discussed.

12/7/61

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12716638 BIOSIS NO.: 199598184471

Purification and Characterization of Honey Sucrase

AUTHOR: Cho Nam-Chul

AUTHOR ADDRESS: Dep. Food Nutr., Dongshin Junior Coll., Kwangju 500-714, South Korea**South Korea

JOURNAL: Korean Biochemical Journal 27 (6): p509-513 1994 1994

ISSN: 0368-4881

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Sucrase (or invertase) (β -D-fructofuranoside fructohydrolase (EC 3.2.1.26)) was **purified** from honey by Acell Plus CM cation exchange chromatography and HPLC-SP column chromatography. The enzyme had a molecular weight of 76,000 daltons, as determined by 10% SDS-PAGE. The enzyme showed activity toward sucrose and maltose but did not catalyze the hydrolysis of lactose, raffinose, melezitose, **inulin**, starch, p-nitrophenyl- α -D-glucopyranoside (α -PNPG), or p-nitrophenyl- β -D-glucopyranoside (β -PNPG). The V_{max} and K_m values of **purified** sucrase against sucrose were 100 U per mg. of **protein** and 91.2 mM, respectively, and against maltose they were 31.25 U per mg. of **protein** and 60 mM, respectively. The optimum pH and temperature of the enzyme were pH 5.0-6.0 and 40-50 degree C, respectively. When **purified** honey sucrase was added to a reaction mixture containing maltose or sucrose, a large amount of monosaccharide was produced, but trisaccharide was not detected. Honey sucrase was inhibited by metal ions and chemical modifiers, such as Hg^{2+} , I^- , 1-fluoro 2,4-dinitrobenzene (FDNB), rho-hydroxymercuribenzoic acid (HMB), and n-bromosuccinimide (NBS), but not by D-fructose, the sucrose hydrolytic product.

12/7/62

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12661325 BIOSIS NO.: 199598129158

%%%Purification%%% and characterization of the enzymes of fructan
biosynthesis in tubers of *Helianthus tuberosus* 'Colombia': I.

Fructan:fructan fructosyl transferase

AUTHOR: Koops Andries J (Reprint); Jonker Harry H

AUTHOR ADDRESS: DLO Cent. Plant Breeding Reproduction Res., CPRO-DLO, PO
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JOURNAL: Journal of Experimental Botany 45 (280): p1623-1631 1994 1994

ISSN: 0022-0957

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Fructan:fructan fructosyl transferase (FFT), one of the enzymes involved in the synthesis of beta-2,1 linked fructose polymers has been %%%purified%%% 205-fold from tubers of *Helianthus tuberosus* harvested in the accumulation phase. The molecular weight of the native as well as the SDS-denatured %%%protein%%% is approximately 70 kDa. On IEF, the %%%protein%%% was separated into five molecular species with pI values between pH 4.5-5.0. The optimum pH for fructosyl transfer activity was between 5.5-7.0. Temperature optimum was in the range of 25-35 degree C; the Q-10 value between 25 and 5 degree C was 1.14. FFT catalysed the self-transfer of fructosyl groups with GF-2, GF-3, GF-4 or GF-5 as substrate and acceptor. The rate of self-transfer with both GF-2 and GF-3 increased linearly with substrate concentration up to 100 mol m⁻³ and was still not saturated at 600 and 300 mol m⁻³, respectively. FFT was unable to hydrolyse GF or to catalyse the self-transfer with GF but could mediate the transfer of fructosyl units from %%%inulin%%% on to GF.

12/7/63

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12494990 BIOSIS NO.: 199497516275

Solubilization, partial %%%purification%%% and functional reconstitution of
a sheep brain endoplasmic reticulum anion channel

AUTHOR: Silvestro A M; Ashley R H (Reprint)

AUTHOR ADDRESS: Dep. Biochem., Univ. Edinburgh, George Square, Edinburgh
EH8 9XD, UK**UK

JOURNAL: International Journal of Biochemistry 26 (9): p1129-1138 1994
1994

ISSN: 0020-711X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: 1. An intracellular anion channel, known to be co-localized in brain endoplasmic reticulum membranes with ryanodine-sensitive calcium-release channels, was incorporated into voltage-clamped planar lipid bilayers from sheep brain microsomal membrane vesicles. 2. Single channels, which displayed a main open-state conductance of 80-100 pS in symmetric 450 mM choline Cl, reduced to approx 20 pS in symmetric 225 mM (choline)-2SO₄ (the solutions also contained 10 mM Tris-HCl, pH 7.4),

discriminated poorly between Cl⁻ and choline⁺ (relative permeability ratio, P-Cl⁻/P-choline⁺, 2.5). 3. Sheep brain microsomal membrane proteins were solubilized in the zwitterionic detergent CHAPS, and subjected to sequential anion-exchange and size-exclusion chromatography; the solubilizate, and partially-purified protein fractions. were then incorporated into large unilamellar liposomes by freeze-thaw sonication. 4. Reconstituted passive anion (Cl⁻)-transport, which was reduced by apprx 60% in the presence of SO₄²⁻, was assayed by measuring the efflux of entrapped ³⁶Cl⁻ (compared to the efflux of (3H)inulin), and also by monitoring the fluorescence quenching of entrapped SPQ by Cl⁻-influx. 5. Cl⁻-transporting activity was enriched up to 200-fold after two stages of purification, and the partially-purified channel protein was incorporated from reconstituted proteoliposomes into planar lipid bilayers, where its permeation behaviour remained very similar to that observed for the native channel.

12/7/64

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12073832 BIOSIS NO.: 199497095117

Purification and some properties of beta-fructofuranosidase from Bifidobacterium adolescentis G1

AUTHOR: Muramatsu Kei; Onodera Shuichi; Kikuchi Masanori; Shiomi Norio (Reprint)

AUTHOR ADDRESS: Dep. Food Sci., Fac. Dairy Sci., Rakuno Gakuen Univ., Ebetsu, Hokkaido 069, Japan**Japan

JOURNAL: Bioscience Biotechnology and Biochemistry 57 (10): p1681-1685 1993 1993

ISSN: 0916-8451

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A unique beta-fructofuranosidase was purified from the extract of Bifidobacterium adolescentis G1 by anion-exchange, hydrophobic, and gel filtration chromatographies, and preparative electrophoresis. The molecular mass was 74 kDa by SDS-PAGE, and the isoelectric point was pH 4.5. The enzyme was a monomeric protein. The pH optimum was at 6.1. The enzyme was stable at pH from 6.5 to 10.0, and up to 45 degree C. The neutral sugar content was 1.2%. The enzyme hydrolyzed 1-kestose faster than sucrose or inulin. The hydrolytic activity was strongly inhibited by Cu²⁺, Ag⁺, Hg⁺, and p-chloromercuribenzoic acid. The K_m (mM) and k_{cat} (s⁻¹) were: 1-kestose, 1.1 and 231; sucrose, 11 and 59.0; inulin, 8.0 and 149, respectively. From the kinetic results, beta-fructofuranosidase from B. adokscentis G1 was concluded to have a high affinity for 1-kestose, thus differing from invertases and exo-inulinases in substrate specificity.

12/7/65

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11984134 BIOSIS NO.: 199497005419

Structural analysis of chicken factor B-like protease and comparison with

mammalian complement proteins factor B and C2
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JOURNAL: Journal of Immunology 151 (8): p4147-4152 1993 1993
ISSN: 0022-1767
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Chicken complement factor B-like protease is a glycoprotein of 95 kDa. Activation of chicken serum complement with **inulin** cleaved the B-like protease into an N-terminal Ba fragment of 37 kDa and a C-terminal Bb fragment of 60 kDa. The whole **protein** and the two fragments were **purified** by affinity chromatography using mAb to chicken Ba or Bb followed by ion exchange chromatography. Amino acid sequencing showed that chicken B-like protease was cleaved at a site homologous to that cleaved in mammalian complement components B and C2 on activation. Limited tryptic digestion of the B-like protease generated fragments similar to Ba and Bb. More than 200 residues of the Ba sequence and two N-linked glycosylation sites were established by amino acid sequencing of peptides derived by digestion with four proteases. Comparison of human and mouse C2 and B sequences indicated a slower evolutionary rate for B (85% sequence identity) than for C2 (74% sequence identity). Comparison of chicken Ba to human and mouse C2b and Ba showed 42 to 45% sequence identity with respect to C2b fragments, and 46 to 49% sequence identity with respect to Ba fragments. Taking the slower evolutionary rate of factor B into account, chicken factor B-like protease seems to be equally related to mammalian complement components B and C2, and the B-like protease most likely represents the present-day descendant of a common ancestral **protein** for mammalian B and C2. This conclusion is in agreement with the requirement for the B-like protease in both classical and alternative activation pathways for chicken complement, and with the apparent lack of a chicken serum **protein** with exclusive C2 activity.

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11899155 BIOSIS NO.: 199396063571
Purification and characterization of an *Aspergillus niger* invertase and its DNA sequence
AUTHOR: Boddy L M; Berges T; Barreau C; Vainstein M H; Dobson M J; Ballance D J; Peberdy J F (Reprint)
AUTHOR ADDRESS: Microbial Biochem. and Genetics Group, Dep. Life Sci., Univ. Nottingham, University Park, Nottingham NG7 2RD, UK**UK
JOURNAL: Current Genetics 24 (1-2): p60-66 1993
ISSN: 0172-8083
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A secreted invertase was **purified** 23-fold by ultrafiltration, ion-exchange, and gel filtration chromatography from the culture supernatant of 18 h sucrose-grown cultures of *Aspergillus niger*.

The %%%purified%%% enzyme hydrolysed sucrose and raffinose but there was no detectable hydrolysis of %%%inulin%%%, melezitose or PNPG. Invertase activity was optimal at pH 5.5 and 50 degree C. The molecular mass of reduced invertase was 115 kDa, as determined by SDS gel electrophoresis. The native molecular weight of between 225 kDa and 250 kDa, estimated by electrophoresis under non-denaturing conditions, suggests that the %%%protein%%% is a dimer of identical subunits. The suc1 gene encoding this %%%protein%%% was completely sequenced. The translated sequence yields a %%%protein%%% of 566 amino acids with a calculated molecular mass of 61 kDa, suggesting that carbohydrates represent about 50% of the mass of the %%%protein%%%.

12/7/67

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11891736 BIOSIS NO.: 199396056152

%%Purification%% and properties of %%%inulin%% fructotransferase (depolymerizing) from Enterobacter sp. S45

AUTHOR: Kang Su-Il; Kim Su-Il (Reprint)

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JOURNAL: Journal of the Korean Agricultural Chemical Society 36 (2): p 105-110 1993

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Korean

ABSTRACT: %%%Inulin%% fructotransferase from Enterobacter sp. S45 was %%%purified%%% with DEAE-cellulose column chromatography and fast %%%protein%%% liquid chromatography. The %%%purified%%% enzyme gave a single band on polyacrylamide gel electrophoresis. The molecular weight was estimated to be 42,800 by SDS-polyacrylamide gel electrophoresis. The optimal pH and temperature for the enzyme reaction were pH 5.5 and 55 degree C, respectively. Mg-2+ activated the enzyme activity, but Fe-3+, Cu-2+, Hg-2+ significantly inhibited. After exhaustive digestion of %%%inulin%% by the enzyme, DFA III, sucrose, 1-kestose and nystose were produced. Sucrose, 1-kestose, raffinose and melezitose can't be used as substrates by the enzyme, but nystose and 1-F-fructofuranosyl nystose were hydrolysed. The Km and Vmax for %%%inulin%% of the enzyme were 1.4 mM and 0.196 mu-mole/min, respectively.

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11880180 BIOSIS NO.: 199396044596

%%Purification%% and characterization of fructan: Fructan

fructosyltransferase from Jerusalem artichoke (Helianthus tuberosus L.)

AUTHOR: Luscher M; Frehner M; Nosberger J

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JOURNAL: New Phytologist 123 (4): p717-724 1993

ISSN: 0028-646X

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Fructan: fructan fructosyltransferase activity (FFT, EC 2.4. 1. 100) from *Helianthus tuberosus* L. was purified 221-fold by a basic procedure involving ammonium sulphate precipitation, lectin chromatography and ion-exchange chromatography. The resulting FFT preparation was separated into three protein bands, of apparent molecular weight 72 800, 60 500 and 56 200, by denaturing polyacrylamide gel electrophoresis. These proteins showed affinity to sucrose-Eupergit. FFT proteins with a molecular weight of 72 800 were isolated by preparative native gel electrophoresis, and yielded six distinguishable forms on separation by analytical isoelectric focusing. Proteins from the basic purification were separated by preparative isoelectric focusing into several forms with isoelectric points between pH 4.3 and 4.5. Samples from the gel with FFT activity were analyzed by denaturing polyacrylamide gel electrophoresis. Three samples contained only protein with the molecular weight 72 800, and one sample contained only protein of apparent molecular weight 60 500. The remaining samples contained a mixture of proteins with molecular weights of 72 800, 60 500 and 56 200. FFT was detected by 1-kestose-dependent nystose production. The enzyme was most active at pH 6.5, and up to 80% of the activity was retained on pre-incubation (1 h) at temperatures of up to 40 degree C. FFT transferred fructosyl groups from oligofructans (degree of polymerization (DP) 3-8) of the inulin series. No glycosyl transfer occurred with 6-kestose, neokestose, maltose, raffinose and maltotriose as the sole substrate. Sucrose efficiently accepted fructosyl units from oligofructans with a K-m of approximately 0.2 mM. The rate of fructosyl transfer increased with degree of polymerization (from DP 4). 1-kestose was shown to be an efficient donor of fructosyl units to sucrose.

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11880141 BIOSIS NO.: 199396044557

Fructan exohydrolase from grasses

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JOURNAL: New Phytologist 123 (3): p453-469 1993

ISSN: 0028-646X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: In grasses, fructan reserves are mobilized from vegetative plant parts during seasonal growth, after defoliation during grazing and from stems during seed filling. Well-illuminated leaves show a diurnal pattern of fructan accumulation during the light and mobilization during the dark. In expanding leaves, fructans are accumulated in cells of the elongation zone and when mobilized are considered to contribute assimilate for synthetic processes. Even in leaves which do not contain high fructan concentrations, high rates of fructan turnover occur. The process of fructan mobilization appears to be regulated in relation to

ontogenic events, demand for assimilate during growth and in response to environmental stress. Hydrolysis of fructans in bacteria is catalyzed by both endo- and exohydrolases. However, in higher plants only fructan exohydrolases (FEH) (EC 3.2.1.80) have been reported. FEH has been extracted from only a limited number of grass species. The pH optimum of FEH activities varies between pH 4.5-5.5, the temperature optimum ranges from 25-40 degree C and FEH is considered to be entirely localized in vacuoles. Estimates of the K-m for FEH assayed using high molecular weight fructan substrates vary widely and should be considered carefully because most substrates are ill-defined. Many studies indicate that crude and partially-purified FEH activity is highest when assayed using a fructan substrate extracted from the species that was the source of the enzyme activity. Inulin extracted from members of the Asteraceae is generally less readily hydrolyzed and levans from bacteria are relatively poor substrates for FEH from grasses. Glycosidic-linkage-specific hydrolysis has been demonstrated for an FEH activity extracted from barley. This FEH activity hydrolyzed beta-2,1-glycosidic linkages more rapidly than beta-2,6-linkages. Most other studies are less conclusive because ill-defined fructan substrates were used. Two isoforms of FEH are reported in leaves of *Lolium* spp., but the roles of isoforms and their kinetic characteristics are not known. FEH activity in different tissues may be regulated by metabolic concentrations, sucrose (5-10 mM) being a strong inhibitor in vitro of FEH from some species. Results of experiments with *Dactylis glomerata* indicate control of expression of FEH activity at the gene level. In stem bases, FEH activity increased after defoliation. The increase was abolished by applications of inhibitors of protein synthesis and was apparently repressed by application of various sugars. Although the rates of fructan hydrolysis measured in vitro are sufficient to explain the in vivo rates of fructan hydrolysis, it is yet to be shown whether fructan hydrolysis in vivo is due to the activity of FEH exclusively, or FEH and invertase-like activities. The overriding conclusion is that the various studies of FEH from grasses present a confusing and incomplete picture of the function, activity and kinetics of this enzyme. This is due in part to the lack of defined, commercially-available substrates. The chromatographic techniques available to most laboratories do not permit purification of sufficient quantities of high molecular weight fructans of specific degree of polymerization, or fructan oligosaccharides with glycosidic linkages which differ from that of the inulin series for enzyme characterization. It is recommended that a few well-defined oligosaccharides be adopted as substrate standards for future research.

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11868374 BIOSIS NO.: 199396032790

Molecular characterization of a fructanase produced by *Bacteroides fragilis* BF-1

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JOURNAL: Journal of Bacteriology 175 (10): p3058-3066 1993

ISSN: 0021-9193

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LANGUAGE: English

ABSTRACT: The *Bacteroides fragilis* BF-1 fructanase-encoding gene (*fruA*) was cloned and expressed in *Escherichia coli* from the recombinant plasmid pBS100. The *fruA* gene consisted of 1,866 bp encoding a protein of 622 amino acids with a calculated M-r of 70,286. The apparent M-r of the fructanase, determined by in vitro cell-free transcription-translation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, was approximately 71,500. An alignment of the amino acid sequences of the *B. fragilis* BF-1 fructanase and the *Bacillus subtilis* levanase revealed that 45.5% of the amino acids were identical. The *fruA* gene was expressed in *E. coli* from its own promoter; however, no *E. coli* promoter-like sequence was evident upstream from the gene. A major *E. coli* transcription start point and a single *B. fragilis* BF-1 transcription start point were located. Expression of the *fruA* gene was constitutive in *E. coli*(pBS100) and *B. fragilis* BF-1. The ratio of sucrase activity to inulinase activity (S/I ratio) was constant for enzyme preparations from *E. coli*(pBS100), indicating that both activities were associated with the fructanase. For *B. fragilis* BF-1, the S/I ratio varied considerably depending on the carbon source used for growth, suggesting that a separate sucrase is produced in addition to the fructanase in *B. fragilis* BF-1. Localization experiments and *TnphoA* mutagenesis indicated that the fructanase was exported to the periplasm. Sequence analysis of the N-terminal region of the fructanase revealed a putative 30-amino-acid signal peptide. The enzymatic properties of the purified fructanase were investigated. The enzyme was able to hydrolyze sucrose, raffinose, inulin, and levan but not melezitose, indicating that it was a beta-D-fructofuranosidase which was able to hydrolyze beta(2 fwardw 1)-linked and beta(2 fwardw 6)-linked fructans.

? ds

Set	Items	Description
S1	9	INULIN AND (REDUCING() SUGAR)
S2	5	INULIN AND TAGATOSE
S3	992	INULIN AND GLUCOSE
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
S5	48	INULIN AND HEMOGLOBIN
S6	8	S5 AND GLUCOSE
S7	0	S5 AND TAGATOSE
S8	0	INULIN AND (PEG?(3)HEMOGLOBIN)
S9	0	INULIN AND (PEG?(3W)HEMOGLOBIN)
S10	0	S1 AND S4
S11	2	S4 AND S5
S12	88	INULIN AND (PURIF? AND PROTEIN)
S13	12	S3 AND (PURIF? AND PROTEIN)

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12/7/1

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0020892799 BIOSIS NO.: 200900233133

Production of beta-fructofuranosidases by *Aspergillus niveus* using agroindustrial residues as carbon sources: Characterization of an intracellular enzyme accumulated in the presence of glucose

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LANGUAGE: English

ABSTRACT: The production of beta-fructofuranosidases by *Aspergillus niveus*, cultivated under submerged fermentation using agroindustrial residues, was investigated. The highest productivity of beta-fructofuranosidases was obtained in Khanna medium supplemented with sugar cane bagasse as carbon source. Glucose enhanced the production of the intracellular enzyme, whereas that of the extracellular one was decreased. The intracellular beta-fructofuranosidase was a trimeric protein of approximately 141 kDa (gel filtration) with 53.5% carbohydrate content, composed of 57 kDa monomers (SDS-PAGE). The optimum temperature and optimum pH were 60 degrees C and 4.5, respectively. The purified enzyme showed good thermal stability and exhibited a half-life of 53 min at 60 degrees C. beta-Fructofuranosidase activity was slightly activated by Cu²⁺, Mn²⁺, Mg²⁺, and Na⁺ at 1 mM concentration. The enzyme hydrolyzed sucrose, raffinose, and inulin, with K_d values of 5.78 mM, 5.74 mM, and 1.74 mM, respectively. (C) 2008 Elsevier Ltd. All rights reserved.

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0020884603 BIOSIS NO.: 200900224937
Purification and biochemical characterization of a native invertase from the hydrogen-producing *Thermotoga neapolitana* (DSM 4359)
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JOURNAL: Extremophiles 13 (2): p345-354 MAR 2009 2009
ITEM IDENTIFIER: doi:10.1007/s00792-008-0222-2
ISSN: 1431-0651
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: This is the first report describing the purification and enzymatic properties of a native invertase (beta-D-fructosidase) in *Thermotogales*. The invertase of the hydrogen-producing thermophilic bacterium *Thermotoga neapolitana* DSM 4359 (hereby named Tni) was a monomer of about 47 kDa having an amino acid sequence quite different from other invertases studied up to now. Its properties and substrate specificity let us classify this protein as a solute-binding protein with invertase activity. Tni was specific for the fructose

moiety and the enzyme released fructose from sucrose and raffinose and the fructose polymer **inulin** was hydrolyzed in an endo-type fashion. Tni had an optimum temperature of 85A degrees C at pH 6.0. At temperatures of 80-85A degrees C, the enzyme retained at least 50% of its initial activity during a 6 h preincubation period. Tni had a K (m) and k (cat) /K (m) values (at 85A degrees C and pH 6.0) of about 14 mM and $5.2 \times 10(8) \text{ M}^{-1} \text{ s}^{-1}$, respectively.

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0020785517 BIOSIS NO.: 200900125851

Purification and Characterization of Extracellular Inulinase from a Marine Yeast *Pichia guilliermondii* and **Inulin** Hydrolysis by the **Purified** Inulinase

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JOURNAL: Biotechnology and Bioprocess Engineering 13 (5): p533-539 SEP-OCT 2008 2008

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DOCUMENT TYPE: Article

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LANGUAGE: English

ABSTRACT: The extracellular inulinase of the marine yeast *Pichia guilliermondii* strain 1 was **purified** to homogeneity resulting in a 7.2-fold increase in specific inulinase activity. The molecular mass of the **purified** enzyme was estimated to be 50.0 kDa. The optimal pH and temperature for the **purified** enzyme were 6.0 and 60 degrees C, respectively. The enzyme was activated by Mn²⁺, Ca²⁺, K⁺, Li⁺, Na⁺, Fe³⁺, Fe²⁺, Cu²⁺, and Co²⁺, but Mg²⁺, Hg²⁺, and Ag⁺ inhibited activity. The enzyme was strongly inhibited by phenylmethanesulphonyl fluoride (PMSF), iodoacetic acid, EDTA, and 1, 10-phenanthroline. The K-m and V-max values of the **purified** inulinase for **inulin** were 21.1 mg/mL and 0.08 mg/min, respectively. A large number of monosaccharides were detected after the hydrolysis of **inulin**. The deduced **protein** sequence from the cloned *P. guilliermondii* strain 1 inulinase gene contained the consensus motifs R-D-P-K-V-F-W-H and W-M-N-D-P-N-G, which are conserved among the inulinases from other microorganisms. (C) KSBB

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0020446718 BIOSIS NO.: 200800493657

The Probiotic *Lactobacillus johnsonii* NCC 533 produces high-molecular-mass **inulin** from sucrose by using an inulosucrase enzyme

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2008 2008
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Fructansucrase enzymes polymerize the fructose moiety of sucrose into levan or *inulin* fructans, with beta(2-6) and beta(2-1) linkages, respectively. The probiotic bacterium *Lactobacillus johnsonii* strain NCC 533 possesses a single fructansucrase gene (open reading frame AAS08734) annotated as a putative levansucrase precursor. However, 13 C nuclear magnetic resonance (NMR) analysis of the fructan product synthesized in situ revealed that this is of the *inulin* type. The *ftf* gene of *L.johnsonii* was cloned and expressed to elucidate its exact identity. The *purified* *L.johnsonii* *protein* was characterized as an inulosucrase enzyme, producing *inulin* from sucrose, as identified by 13 C NMR analysis. Thin-layer chromatographic analysis of the reaction products showed that InuJ synthesized, besides the *inulin* polymer, a broad range of fructose oligosaccharides. Maximum InuJ enzyme activity was observed in a pH range of 4.5 to 7.0, decreasing sharply at pH 7.5. InuJ exhibited the highest enzyme activity at 55 degrees C, with a drastic decrease at 60 degrees C. Calcium ions were found to have an important effect on enzyme activity and stability. Kinetic analysis showed that the transfructosylation reaction of the InuJ enzyme does not obey Michaelis-Menten kinetics. The non-Michaelian behavior of InuJ may be attributed to the oligosaccharides that were initially formed in the reaction and which may act as better acceptors than the growing polymer chain. This is only the second example of the isolation and characterization of an inulosucrase enzyme and its *inulin* (oligosaccharide) product from a *Lactobacillus* strain. Furthermore, this is the first *Lactobacillus* strain shown to produce *inulin* polymer in situ.

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0020205604 BIOSIS NO.: 200800252543
Exo-inulinase of *Aspergillus niger* N402: A hydrolytic enzyme with significant transfructosylating activity
AUTHOR: Goosen C; Van der Maarel M J E C (Reprint); Dijkhuizen L
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JOURNAL: Biocatalysis and Biotransformation 26 (1-2): p49-58 2008 2008
ITEM IDENTIFIER: doi:10.1080/10242420701806686
ISSN: 1024-2422
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The *purified* exo-inulinase enzyme of *Aspergillus niger* N402

(AngInuE; heterologously expressed in *Escherichia coli*) displayed a sucrose:inulin (S/I) hydrolysis ratio of 2.3, characteristic for a typical exo-inulinase. The enzyme also had significant transfructosylating activity with increasing sucrose concentrations, producing various oligosaccharides. The AngInuE protein molecular mass was 57 kDa, close to the calculated value for the mature protein. AngInuE thus was active as a monomeric, non-glycosylated protein. Contradictory data on hydrolysis/transfructosylation activity ratios have been published for the (almost) identical (but monomeric or dimeric and glycosylated) exo-inulinases of other aspergilli. Our data clearly show that the AngInuE enzyme, produced in and purified from *E. coli*, is a broad specificity exo-inulinase that also has significant transfructosylating activity with sucrose. Analysis of site-directed mutants of AngInuE showed that the glycoside hydrolase family 32 conserved domain G is important for catalytic efficiency, with a clear role in hydrolysis of both sucrose and fructans.

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0019985793 BIOSIS NO.: 200800032732

The source of fermentable carbohydrates influences the in vitro

protein synthesis by colonic bacteria isolated from pigs

AUTHOR: Bindelle J (Reprint); Buidgen A; Wavreille J; Agneessens R; Destain J P; Wathelet B; Leterme P

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JOURNAL: Animal 1 (8): p1126-1133 SEP 2007 2007

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ABSTRACT: Two in vitro experiments were carried out to quantify the incorporation of nitrogen (N) by pig colonic bacteria during the fermentation of dietary fibre, including non-starch polysaccharides and resistant starch. In the first experiment, five purified carbohydrates were used: starch (S), cellulose (C), inulin (I), pectin (P) and xylan (X). In the second experiment, three pepsin-pancreatin hydrolysed ingredients were investigated: potato, sugar-beet pulp and wheat bran. The substrates were incubated in an inoculum, prepared from fresh faeces of sows and a buffer solution providing N-15-labelled NH₄Cl. Gas production was monitored. Bacterial N incorporation (BNI) was estimated by measuring the incorporation of 15 N in the solid residue at half-time, to asymptotic gas production (T/2). The remaining substrate was analysed for sugar content. Short-chain fatty acids (SCFA) were determined in the liquid phase. In the first experiment, the fermentation kinetics differed between the substrates. P, S and I showed higher rates of degradation ($P < 0.001$), while X and C showed a longer lag time and T/2. The sugar disappearance reached 0.91, 0.90, 0.81, 0.56 and 0.46, respectively, for P, I, S, C and X. Among them, S and I fixed more N per gram substrate ($P < 0.05$) than C, X and P (22.9 and 23.2 mg fixed N per gram fermented substrate v. 11.3, 12.3 and 9.8,

respectively). Production of SCFA was the highest for the substrates with low N fixation: 562 and 565 mg/g fermented substrate for X and C v. 290 to 451 for P, I and S ($P < 0.01$). In the second experiment, potato and sugar-beet pulp fermented more rapidly than wheat bran ($P < 0.001$). Substrate disappearance at T/2 varied from 0.17 to 0.50. BNI were 18.3, 17.0 and 10.2 fixed N per gram fermented substrate, for sugar-beet pulp, potato and wheat bran, respectively, but were not statistically different. SCFA productions were the highest with wheat bran (913 mg/g fermented substrate) followed by sugar-beet pulp (641) and potato (556) ($P < 0.05$). The differences in N uptake by intestinal bacteria are linked to the partitioning of the substrate energy content between bacterial growth and SCFA production. This partitioning varies according to the rate of fermentation and the chemical composition of the substrate, as shown by the regression equation linking BNI to T/2 and SCFA ($r^2 = 0.91$, $P < 0.01$) and the correlation between BNI and insoluble dietary fibre ($r = -0.77$, $P < 0.05$) when pectin was discarded from the database.

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0019974616 BIOSIS NO.: 200800021555

Production and characterization of a thermostable extracellular beta-D-fructofuranosidase produced by *Aspergillus ochraceus* with agroindustrial residues as carbon sources

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ABSTRACT: The filamentous fungus *Aspergillus ochraceus* produced high levels of a thermostable extracellular P-D-fructofuranosidase (EC 3.2.1.26) when cultured for 96 h, at 40 degrees C, in Khanna medium supplemented with sugar cane bagasse as carbon source. The enzyme was 7.1-fold purified, with a recovery of 24%, by two chromatographic steps in DEAE-cellulose and Sephacryl S-200. The purified enzyme was homogeneous according to electrophoretic criteria. P-D-Fructofuranosidase was a homodimeric glycoprotein with 41% carbohydrate content and apparent molecular mass of 135 kDa, estimated by gel filtration in Sephacryl S-200, or 79 kDa by SDS-PAGE. Optima of pH and temperature were 4.5 and 60 degrees C, respectively. The enzyme showed a t_{50} of 60 min at 60 degrees C. The enzyme activity was stimulated by Mn^{2+} (57%), Mg^{2+} (50%), Na^+ (35%) and Ba^{2+} (20%), and inhibited by Cu^{2+} and Hg^{2+} . Glucose at 40 mM stimulated the *A. ochraceus* extracellular beta-fructofuranosidase in about 2.68-fold. The enzyme hydrolyzed raffinose, sucrose and inulin, exhibiting K_m of 7.37, 13.4 and 2.66 mM, and V_{max} of 22.39, 42.13 and 3.14 U mg⁻¹ protein⁻¹, respectively. Transfructosylation reactions were not detected, since glucose and

fructose were the only products from sucrose hydrolysis. (C) 2007 Elsevier Inc. All rights reserved.

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0019943376 BIOSIS NO.: 200700603117

Regulation of the interactions of pp2a and ppl with occludin during the assembly of tight junctions (TJ): Pp2a dephosphorylates occludin predominantly on phospho-threonine, while ppl dephosphorylates PhosphoSerine

AUTHOR: Seth Ankur; Rao Radhakrishna r

JOURNAL: Gastroenterology 132 (4, Suppl. 2): pA137-A138 APR 2007 2007

CONFERENCE/MEETING: Digestive Disease Week Meeting/108th Annual Meeting of the American-Gastroenterological-Association Washington, DC, USA May 19-24, 2007; 20070519

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RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Occludin, a transmembrane protein of TJ, is phosphorylated on Ser/Thr residues. Our previous study indicated that PP2A and PPI negatively regulate the assembly of TJ in Caco2 cells. In this study we show that the interaction of PP2A and PPI with occludin is regulated during the assembly of TJ, and that PP2A and PPI selectively dephosphorylate occludin on Ser or Thr. Methods: Caco-2 cells were transfected with antisense oligos to PP2A (ASPP2A), PPI (AS-PPI) or missense oligo (MS-oligo). TJ assembly was assessed by calcium switch method, and monitored by measuring TER, FITC-inulin flux and confocal microscopy of occludin and ZO-1. Phosphorylation of occludin was evaluated by immunoprecipitation of p-Ser and p-Thr and immunoblot analysis. Association of PP2A and PPI with occludin was determined by co-immunoprecipitation, and by pair wise binding using GST-Occludin-C (C-terminal 150 A.a.) and purified PP2A and PPI. Interaction of GST-Occludin-C with PP2A, PPI and PKC zeta was evaluated by GST pull down assay using extracts from cells at different stages of calcium switch. To evaluate occludin-dephosphorylation, immunocomplexes of phospho-occludin was incubated with PP2A or PPI and immunoblotted for pThr and p-Ser. Results: Calcium depletion by EGTA reduced TER, increased inulin flux, and induced redistribution of occludin and ZO-1 from the junctions. Calcium gradually increased TER, reduced inulin flux and organized occludin and ZO-1 at the junctions. Calcium depletion dramatically reduced Thr-phosphorylation of occludin, while it was elevated during calcium-induced reassembly of TJ. Co-immunoprecipitation of PP2A and PPI with occludin was greater in EGTA-treated cells, but it was gradually reduced during the reassembly. GST pull down assay showed that the interaction of occludin with PP2A and PPI, but not PKC, was greater in EGTA-treated cells, while it was reduced during the assembly of TJ. Incubation of phospho-occludin with PP2A for 10 min reduced the level of p-Thr without affecting p-Ser, while incubation with PPI reduced p-Ser

without affecting p-Thr. Transfection of AS-PP2A or AS-PPI accelerated the reassembly of TJ. In AS-PP2A-transfected cells, the level of Thr-phosphorylated occludin during TJ-reassembly was significantly greater compared to MS-oligo-transfected cells. In AS-PPI-transfected cells however, Ser-phosphorylated occludin was increased during the reassembly of TJ, while it was slightly reduced in AS-PP2A or MS-oligo-transfected cells. Conclusion: These results suggest that PP2A and PPI may have distinct roles in regulating the phosphorylation of occludin on Thr/Ser residues and TJ integrity.

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0019849807 BIOSIS NO.: 200700509548

Influence of **purified** dietary fibre on bacterial **protein** synthesis in the large intestine of pigs, as measured by the gas production technique

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JOURNAL: Livestock Science 109 (1-3, Sp. Iss. SI): p232-235 MAY 15 2007 2007

ITEM IDENTIFIER: doi:10.1016/j.livsci.2007.01.126

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LANGUAGE: English

ABSTRACT: Microbial fermentation of non-digestible carbohydrates in the pig's large intestine induces a shift of N excretion from urea in urine to bacterial **protein** in faeces. Experiments were carried out to measure the mineral N incorporation by the pig intestinal microflora using 5 **purified** carbohydrates in a gas-test: starch (S), cellulose (C), **inulin** (I), pectin (P) and xylan (X). Fermentation kinetics was modelled. N source in the buffer solution was replaced by N-15 labelled NH₄Cl. The bacterial N fixation was determined at mid-fermentation, measuring 15 N incorporation into the solid phase of the buffer. The bacterial N fixation was higher ($P < 0.001$) with I and S (19.9 and 18.1mg N/g incubated DM), compared to P, C and X (8.7, 5.9 and 5.5 respectively). **Inulin** and S were fermented also more rapidly, even if (0.081 h⁻¹) and C (0.074 h⁻¹) showed lower half time fractional rate of degradation than S (0.153 h⁻¹), P (0.133 h⁻¹) and X (0.104 h⁻¹). The insoluble dietary fibre content of the substrates was negatively correlated to bacterial N fixation ($r = -0.957$, $P = 0.011$). The high crude **protein** content of P (32.5 mg g⁻¹DM) might explain the lower impact of this substrate on bacterial N fixation, despite its rapid fermentation. Beside the proportion of insoluble fibre, the N content and the rate of fermentation seem to be the major factors influencing bacterial **protein** synthesis. Further studies including ingredients with variable content of indigestible **protein** and mean retention time in the pig's intestines are necessary. (c) 2007 Elsevier B.V. All rights reserved.

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0019727694 BIOSIS NO.: 200700387435

%%Protein%% phosphatases 2A and 1 interact with occludin and negatively regulate the assembly of tight junctions in the CACO-2 cell monolayer

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LANGUAGE: English

ABSTRACT: Occludin is hyperphosphorylated on Ser and Thr residues in intact epithelial tight junction (TJ); however, the role of this phosphorylation in the assembly of TJ is unclear. The influence of %%protein%% phosphatases PP2A and PP1 on the assembly of TJ and phosphorylation of occludin was evaluated in Caco-2 cells. %%Protein%% phosphatase inhibitors and reduced expression of PP2A-C alpha and PP1 alpha accelerated the calcium-induced increase in transepithelial electrical resistance and barrier to %%inulin%% permeability and also enhanced the junctional organization of occludin and ZO-1 during TJ assembly. Phosphorylation of occludin on Thr residues, but not on Ser residues, was dramatically reduced during the disassembly of TJ and was gradually increased during the reassembly. PP2A and PP1 co-immunoprecipitate with occludin, and this association was reduced during the assembly of TJ. Glutathione S-transferase (GST) pull-down assay using recombinant GST-occludin demonstrated that cellular PP2A and PP1 bind to the C-terminal tail of occludin, and these interactions were also reduced during the assembly of TJ. Apairwise binding assay using GST- occludin and %%purified%% PP2A and PP1 demonstrates that PP2A and PP1 directly interacts with the C-terminal tail of occludin. In vitro incubation of phospho- occludin with PP2A or PP1 indicated that PP2A dephosphorylates occludin on phospho- Thr residues, whereas PP1 dephosphorylates it on phospho- Ser. This study shows that PP2A and PP1 directly interact with occludin and negatively regulate the assembly of TJ by modulating the phosphorylation status of occludin.

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0019717242 BIOSIS NO.: 200700376983

Molecular and biochemical characterization of a novel intracellular invertase from Aspergillus niger with transfructosylating activity

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JOURNAL: Eukaryotic Cell 6 (4): p674-681 APR 2007 2007
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ABSTRACT: A novel subfamily of putative intracellular invertase enzymes (glycoside hydrolase family 32) has previously been identified in fungal genomes. Here, we report phylogenetic, molecular, and biochemical characteristics of SucB, one of two novel intracellular invertases identified in *Aspergillus niger*. The sucB gene was expressed in *Escherichia coli* and an invertase-negative strain of *Saccharomyces cerevisiae*. Enzyme %purified% from *E. coli* lysate displayed a molecular mass of 75 kDa, judging from sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. Its optimum pH and temperature for sucrose hydrolysis were determined to be 5.0 and 37 to 40 degrees C, respectively. In addition to sucrose, the enzyme hydrolyzed 1-kestose, nystose, and raffinose but not %inulin% and levan. SucB produced 1-kestose and nystose from sucrose and 1-kestose, respectively. With nystose as a substrate, products up to a degree of polymerization of 4 were observed. SucB displayed typical Michaelis-Menten kinetics with substrate inhibition on sucrose (apparent K_m, K_i, and V_{max} of 2.0 +/- 0.2 mM, 268.1 +/- 18.1 mM, and 6.6 +/- 0.2 μ mol min⁻¹ mg⁻¹ of %protein% [total activity], respectively). At sucrose concentrations up to 400 mM, transfructosylation (FTF) activity contributed approximately 20 to 30% to total activity. At higher sucrose concentrations, FTF activity increased to up to 50% of total activity. Disruption of sucB in *A. niger* resulted in an earlier onset of sporulation on solid medium containing various carbon sources, whereas no alteration of growth in liquid culture medium was observed. SucB thus does not play an essential role in %inulin% or sucrose catabolism in *A. niger* but may be needed for the intracellular conversion of sucrose to fructose, glucose, and small oligosaccharides.

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0019507421 BIOSIS NO.: 200700167162
Cloning, expression, and characterization of *Bacillus* sp. snu-7 %inulin% fructotransferase
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JOURNAL: Journal of Microbiology and Biotechnology 17 (1): p37-43 JAN 2007 2007
ISSN: 1017-7825_(print) 1738-8872_(electronic)
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LANGUAGE: English

ABSTRACT: A gene encoding **inulin** fructotransferase (di-D-fructofuranose 1,2': 2,3' dianhydride [DFA III]-producing IFTase, EC 4.2.2.18) from *Bacillus* sp. snu-7 was cloned. This gene was composed of a single, 1,353-bp open reading frame encoding a **protein** composed of a 40-amino acid signal peptide and a 410-amino acid mature **protein**. The deduced amino acid sequence was 98% identical to *Arthrobacter globiformis* C11-1 IFTase (DFA M-producing). The enzyme was successfully expressed in *E. coli* as a functionally active, His-tagged **protein**, and it was **purified** in a single step using immobilized metal affinity chromatography. The **purified** enzyme showed much higher specific activity (1,276 units/mg **protein**) than other DFA III-producing IFTases. The recombinant and native enzymes were optimally active in very similar pH and temperature conditions. With a 103-min half-life at 60 degrees C, the recombinant enzyme was as stable as the native enzyme. Acidic residues and cysteines potentially involved in the catalytic mechanism are proposed based on an alignment with other IFTases and a DFA IIIase.

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19368275 BIOSIS NO.: 200700028016

Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells

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ABSTRACT: Prebiotic oligosaccharides are thought to provide beneficial effects in the gastrointestinal tract of humans and animals by stimulating growth of selected members of the intestinal microflora. Another means by which prebiotic oligosaccharides may confer health benefits is via their antiadhesive activity. Specifically, these oligosaccharides may directly inhibit infections by enteric pathogens due to their ability to act as structural mimics of the pathogen binding sites that coat the surface of gastrointestinal epithelial cells. In this study, the ability of commercial prebiotics to inhibit attachment of microcolony-forming enteropathogenic *Escherichia coli* (EPEC) was investigated. The adherence of EPEC strain E2348/69 on HEp-2 and Caco-2 cells, in the presence of fructooligosaccharides, **inulin**, galactooligosaccharides (GOS), lactulose, and raffinose was determined by cultural enumeration and microscopy. **Purified** GOS exhibited the greatest adherence inhibition on both HEp-2 and Caco-2 cells, reducing the adherence of EPEC by 65 and 70%, respectively. In addition, the average number of bacteria per microcolony was significantly reduced from 14 to 4 when GOS was present. Adherence inhibition by GOS was dose dependent, reaching a maximum at 16 mg/ml. When GOS was added to adhered EPEC cells, no displacement was observed. The expression of BfpA, a

bundle-forming-pilus %%protein%% involved in localized adherence, was not affected by GOS, indicating that adherence inhibition was not due to the absence of this adherence factor. In addition, GOS did not affect autoaggregation. These observations suggest that some prebiotic oligosaccharides may have antiadhesive activity and directly inhibit the adherence of pathogens to the host epithelial cell surface.

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19080483 BIOSIS NO.: 200600425878

Isolation and characterization of a novel lectin from the mushroom

Armillaria luteo-virens

AUTHOR: Feng K; Liu Q H; Ng T B; Liu H Z; Li J Q; Chen G; Sheng H Y; Xie Z L; Wang H X (Reprint)

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JOURNAL: Biochemical and Biophysical Research Communications 345 (4): p 1573-1578 JUL 14 2006 2006

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ABSTRACT: From the dried fruiting bodies of the Mushroom *Armillaria luteo-virens*, a dimeric lectin with a molecular mass of 29.4 kDa has been isolated. The %%purification%% procedure involved (NH₄)₂SO₄ precipitation. ion exchange chromatography oil DEAE-cellulose, CM-cellulose, and Q-Sepharose, and gel filtration by fast %%protein%% liquid chromatography on Superdex 75. The hemagglutinating activity of the lectin could not be inhibited by simple sugars but was inhibited by the polysaccharide %%inulin%%. The activity was stable up to 70 degrees C but was acid and alkali-labile. Salts including FeCl₃, AlCl₃, and ZnCl₂ inhibited the activity whereas MgCl₂, MnCl₂. and CaCl₂ did not. The lectin stimulated mitogenic response Of mouse splenocytes with the maximal response achieved by 1 mu M lectin. Proliferation of tumor cells including MBL2 cells, HeLa cells, and L1210 cells was inhibited by the lectin with an IC₅₀ of 2.5, 5, and 10 mu M, respectively. However, proliferation of HepG2 cells was not affected. The novel aspects of the isolated lectin include a novel N-terminal sequence, fair thermostability, acid stability, and alkali stability, together with potent mitogenic activity toward spleen cells and antiproliferative activity toward tumor cells. (c) 2006 Elsevier Inc. All rights reserved.

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18995815 BIOSIS NO.: 200600341210

%%Purification%% and properties of a heat-stable exoinulinase isoform from *Aspergillus fumigatus*

AUTHOR: Gill Prabhjot Kaur; Manhas Rajesh Kumari; Singh Prabhjeet (Reprint)

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JOURNAL: Bioresource Technology 97 (7): p894-902 MAY 2006 2006
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ABSTRACT: An inducible extracellular exoinulinase (isoform II) was
%%purified%% from the extracellular extract of *Aspergillus fumigatus* by
ammonium sulphate precipitation, followed by successive chromatographies
oil DEAE-Sephacel, Octyl-Sepharose (HIC) Sephacryl S-200, affinity
chromatography on ConA-CL Agarose and Sephacryl S-100 columns. The enzyme
was %%purified%% 75-folds with 3.2%, activity yield from the starting
culture broth. The %%purified%% isoform II was a monomeric 62 kDa
%%protein%% with a pI value of 4.5. The enzyme showed maximum activity
at pH 6.0 and was stable over a pH range of 4.0-7.0, whereas the optimum
temperature for enzyme activity was 60 degrees C. The inulinase isoform
II showed exo-inulinolytic activity and retained 72% and 44% residual
activity after 12 h at 60 degrees C and 70 degrees C, respectively. The
%%inulin%% hydrolysis activity was completely abolished with 5 mM Hg2+
and Fe2+, whereas K+ and Cu2+ enhanced the inulinase activity. As
compared to sucrose, stachyose and raffinose the %%purified%% enzyme
had a lower K-m(1.25 mM) and higher catalytic center activity (K-cat =
3.47 x 10(4) min(-1)) for %%inulin%%. As compared to exoinulinase
isoform I of *A. fumigatus*, %%purified%% earlier, the isoform II is more
thermostable and is a potential candidate for commercial production of
fructose from %%inulin%%. (c) 2005 Elsevier Ltd. All rights reserved.

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18970528 BIOSIS NO.: 200600315923
%%Purification%% and biochemical properties of a new thermostable
xylanase from symbiotic fungus, *Termitomyces* sp.
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JOURNAL: African Journal of Biotechnology 5 (3): p273-282 FEB 2 2006 2006
ISSN: 1684-5315
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LANGUAGE: English

ABSTRACT: A xylanase was %%purified%% from symbiotic fungus, *Termitomyces*
sp. by chromatography on columns of DEAE-Sepharose, CM-Sepharose, gel
filtration and Phenyl-Sepharose. The preparation was shown to be
homogenous by polyacrylamide gel electrophoresis. The %%purified%%
enzyme displayed two %%protein%% bands on SDS-polyacrylamide gel
electrophoresis and its molecular mass was estimated to 80-87 kDa. The
xylanase exhibited maximum activity at 65-70 degrees C and at pH 5.6, but
it retained more than 80% of its activity in the pH range 5.0-6.0. The

enzyme was stable for a long time-period up to 5.0 degrees C and for 1 h at 60 degrees C. Although the xylanase had a lower carboxymethylcellulase activity, it lacked activity towards substituted xylan, xylobiose, **inulin**, starch, polygalacturonic acid or pNP-glycosides. Kinetic parameters indicated higher efficiency in the hydrolysis of beechwood xylan and birchwood xylan. The xylanase activity was stimulated by K⁺, Mn²⁺ and dithiol-reducing agents and was sensitive to Cu²⁺, Fe²⁺, Zn²⁺ and detergent agents. The enzymatic activity was observed in presence of urea up to a 1% (w/v) concentration. The enzyme could also be used in the presence of organic solvents such as acetone or dioxane (5%, v/v) without loss of activity.

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18922427 BIOSIS NO.: 200600267822

Highly **purified** lipoteichoic acid from gram-positive bacteria induces in vitro blood-brain barrier disruption through glia activation: Role of pro-inflammatory cytokines and nitric oxide

AUTHOR: Boveri M; Kinsner A; Berezowski V; Lenfant A-M; Draing C; Cecchelli R; Dehouck M-P; Hartung T; Prieto P; Bal-Price A (Reprint)

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JOURNAL: Neuroscience 137 (4): p1193-1209 2006 2006

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ABSTRACT: The co-culture of bovine brain capillary endothelial cells and rat primary glial cells was established as an in vitro blood-brain barrier model to investigate the mechanisms by which the Gram-positive bacterial cell wall components lipoteichoic acid and muramyl dipeptide induced injury of blood-brain barrier structure and function. We found that highly **purified** lipoteichoic acid disrupted blood-brain barrier integrity in a concentration- and time-dependent manner indirectly, through glia activation. Low trans-endothelial electrical resistance and high permeability to fluorescein isothiocyanate-**inulin** observed in the presence of lipoteichoic acid-activated glial cells were potentiated by muramyl dipeptide and could be reversed only when glial cells were activated by lipoteichoic acid at 10 µg/ml but not with a higher lipoteichoic acid concentration (30 µg/ml). Immunocytochemistry analysis revealed no evident changes in the distribution of the cytoskeleton **protein** F-actin and tight junction proteins occludin and claudin after lipoteichoic acid treatment. However, the tight junction associated **protein** AHNK clearly revealed the morphological alteration of the endothelial cells induced by lipoteichoic acid. Lipoteichoic acid-activated glial cells produced nitric oxide and pro-inflammatory cytokines (tumor necrosis factor-α and interleukin-1β) that contributed to lipoteichoic acid-induced blood-brain barrier disruption, since the direct treatment of the endothelial monolayer with tumor necrosis factor-α or interleukin-1β increased blood-brain barrier permeability, whereas the pre-treatment of lipoteichoic acid-activated glial cells with antibodies against these two cytokines

blocked lipoteichoic acid effects. Additionally, nitric oxide was also involved in blood-brain barrier damage, since the nitric oxide donor itself (diethylenetriamine-nitric oxide adduct) increased blood-brain barrier permeability and inducible nitric oxide synthase inhibitor (1400W) partially reversed lipoteichoic acid-induced trans-endothelial electrical resistance decrease. (c) 2005 Published by Elsevier Ltd on behalf of IBRO.

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18822441 BIOSIS NO.: 200600167836

%%Purification%%, cloning and functional characterization of a fructan 6-exohydrolase from wheat (*Triticum aestivum* L.)

AUTHOR: Van Riet Liesbet; Nagaraj Vinay; Van den Ende Wim; Clerens Stefan; Wiemken Andres; Van Laere Andre (Reprint)

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ABSTRACT: Fructans, beta 2-1 and/or beta 2-6 linked polymers of fructose, are produced by fructosyltransferases (FTs) from sucrose. They are important storage carbohydrates in many plants. Fructan reserves, widely distributed in plants, are believed to be mobilized via fructan exohydrolases (FEHs). The %%purification%%, cloning, and functional characterization of a 6-FEH from wheat (*Triticum aestivum* L.) are reported here. It is the first FEH shown to hydrolyse exclusively beta 2-6 bonds found in a fructan-producing plant. The enzyme was %%purified%% to homogeneity using ammonium sulphate precipitation, ConA affinity-, ion exchange-, and size exclusion chromatography and yielded a single band of 70 kDa following SDS-PAGE. Sequence information obtained by mass spectrometry of in-gel trypsin digests demonstrated the presence of a single %%protein%%. Moreover, these unique peptide sequences, together with some ESTs coding for them, could be used in a RT-PCR based strategy to clone a 1.7 kb cDNA. Functionality tests of the cDNA performed after heterologous expression in the yeast *Pichia pastoris* showed-as did the native enzyme from wheat-a very high activity of the produced %%protein%% against bacterial levan, 6-kestose, and phlein whilst sucrose and %%inulin%% were not used as substrates. Therefore the enzyme is a genuine 6-FEH. In contrast to most FEHs from fructan-accumulating plants, this FEH is not inhibited by sucrose. The relative abundance of 6-FEH transcripts in various tissues of wheat was investigated using quantitative RT-PCR.

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18273609 BIOSIS NO.: 200500180674

Production of inulooligosaccharides by endoinulinases from *Aspergillus ficuum*

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RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Inulooligosaccharide (IOS) production from inulin was studied using a partially purified endoinulinase and a purified endoinulinase, which originated from *Aspergillus ficuum*. At the optimal conditions, including 50 g/L inulin, an enzyme concentration of 10 U/g substrate, 45 degreeC, and pH 6.0, the inulin-degrading degree by partially purified endoinulinase was 74% and an IOS yield over 50% were observed after 72 h. The major products were identified as DP2 to DP4. The purified Endo-I was used for inulo-oligo-saccharide production at the optimal conditions obtained with orthogonal experiments, including pH 5.0, 45 degreeC, 50 g/L inulin, and an enzyme concentration of 10 U/g substrate. With pure inulin as substrate, the maximum inulin-hydrolyzing degree was 75% and the total IOS yield was 70% after 72 h. The hydrolysis products consisted of DP2 to DP8 with HPLC, and DP3 and DP4 were relatively high. With Jerusalem artichoke juice as substrate, the inulin-hydrolyzing extent reached 89% and the maximum IOS production was up to 80% after 72 h. Various IOS with different DP (mainly DP2, DP3, DP4, DP5, DP7, DP8) were evenly distributed in the final reaction products. Copyright 2004 Elsevier Ltd. All rights reserved.

12/7/20

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18245394 BIOSIS NO.: 200500152459

Purification and characterization of a fructosyltransferase from onion bulbs and its key role in the synthesis of fructo-oligosaccharides in vivo

AUTHOR: Fujishima Masaki; Sakai Hideki; Ueno Keiji; Takahashi Natsuko; Onodera Shuichi; Benkeblia Nouredine; Shiomi Norio (Reprint)

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JOURNAL: New Phytologist 165 (2): p513-524 February 2005 2005

MEDIUM: print

ISSN: 0028-646X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A fructosyltransferase that transfers the terminal (2 fwardar 1)-beta-linked D-fructosyl group of fructo-oligosaccharides

(1F(1-beta-D-fructofuranosyl)n sucrose, n 1) to HO-6 of the glucosyl residue and HO-1 of the fructosyl residue of similar saccharides (1F(1-beta-D-fructofuranosyl)m sucrose, m 0) has been purified from an extract of the bulbs of onion (*Allium cepa*). Successive column chromatography using DEAE-Sepharose CL-6B, Toyopearl HW65, Toyopearl HW55, DEAE-Sepharose CL-6B (2nd time), Sephadex G-100, Concanavalin A Sepharose, and Toyopearl HW-65 (2nd time) were applied for protein purification. The general properties of the enzyme, were as follows: molecular masses of 66 kDa (gel filtration chromatography), and of 52 kDa and 25 kDa (SDS-PAGE); optimum pH of c. 5.68, stable at 20-40degreeC for 15 min; stable in a range of pH 5.30-6.31 at 30degreeC for 30 min, inhibited by Hg2+, Ag+, p-chloromercuribenzoic acid (p-CMB) and sodium dodecyl sulfate (SDS), activated by sodium deoxycholate, Triton X-100 and Tween-80. The amino acid sequence of the N-terminus moiety of the 52-kDa polypeptide was ADNEFPWTNDMLAWQRCGFHFRTVRYNMNDPSGPMYYKGWYHLFYQHKNKDFAYXG and the amino acid sequence from the N-terminus of the 25-kDa polypeptide was ADVGYXCSTSGGAATRGTLGPFGLL VLANQDLTENTATYFYVSKGTDGALRTHFCQDET. The enzyme tentatively classified as fructan: fructan 6G-fructosyltransferase (6G-FFT). The enzyme is proposed to play an important role in the synthesis of inulin and inulinneo-series fructo-oligosaccharides in onion bulbs.

12/7/21

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18164997 BIOSIS NO.: 200500072062

Inulin-derived adjuvants efficiently promote both Th1 and Th2 immune responses

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JOURNAL: Immunology and Cell Biology 82 (6): p611-616 December 2004 2004

MEDIUM: print

ISSN: 0818-9641

DOCUMENT TYPE: Article; Literature Review

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: There has been a recent resurgence of interest into new and improved vaccine adjuvants. This interest has been stimulated by the need for new vaccines to combat problematic pathogens such as SARS and HIV, and to counter potential bioterrorist attacks. A major bottleneck in vaccine development is the low immunogenicity of purified subunit or recombinant proteins, creating the need for safe human adjuvants with high potency. A major problem in the search for the ideal adjuvant is that adjuvants that promote cell-mediated (Th1) immunity (e.g. Freund's complete adjuvant) generally have unacceptable local or systemic toxicity that precludes their use in human vaccines. There is a need for a safe, non-toxic adjuvant that is able to stimulate both cell-mediated and humoral immunity. Inulin-derived adjuvants that principally stimulate the innate immune system through their ability to activate the alternative complement pathway have proven ability to induce both cellular and humoral immunity. With their excellent tolerability, long

shelf-life, low cost and easy manufacture, they offer great potential for use in a broad range of prophylactic and therapeutic vaccines. Based on successful animal studies in a broad range of species, human trials are about to get underway to validate the use of inulin-based adjuvants in prophylactic vaccines against hepatitis B, malaria and other pathogens. If such trials are successful, then it is possible that inulin-derived adjuvants will one day replace alum as the adjuvant of choice in most human prophylactic vaccines.

12/7/22

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18086156 BIOSIS NO.: 200400467385

Purification and characterization of inulinase from *Aspergillus niger* AF10 expressed in *Pichia pastoris*

AUTHOR: Zhang Linghua ' (Reprint); Zhao Changxin; Zhu Daochen; Ohta Yoshiyuki; Wang Yunji

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JOURNAL: Protein Expression and Purification 35 (2): p272-275 June 2004 2004

MEDIUM: print

ISSN: 1046-5928

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The inuA1 gene encoding an exoinulinase from *Aspergillus niger* AF10 was expressed in *Pichia pastoris*, and the recombinant enzyme activity was 316 U/ml in a 5 L fermentor, with the inulinase protein accounting for 35% of the total protein of fermentation broth. The hydrolysis rate of mulin can reach 92%, with a 25 U/g inulin enzyme addition, and 90% of fructose content after 6 h. Glucose can significantly inhibit the enzymatic hydrolysis of inulin. This is the first report of glucose inhibition of inulinase-catalyzed hydrolysis. Copyright 2004 Elsevier Inc. All rights reserved.

12/7/23

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17929645 BIOSIS NO.: 200400300402

Cloning, expression, and purification of exoinulinase from *Bacillus* sp. snu-7

AUTHOR: Kim Kyoung-Yun; Koo Bong-Seong; Jo Dohyun; Kim Su-Il (Reprint)

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JOURNAL: Journal of Microbiology and Biotechnology 14 (2): p344-349 April 2004 2004

MEDIUM: print

ISSN: 1017-7825

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A gene encoding inulin-degrading enzyme of *Bacillus* sp. snu-7 with ORF of 1536 nucleotides was cloned. And it was overexpressed as His-tagged protein in *E. coli* BL21 (DE3) pLysS using pRSET B vector containing mature enzyme sequence. Maximum enzyme production was achieved by IPTG (0.1 mM) induction at OD₆₀₀ 1.2 and 30°C, followed by 6 h incubation. The expressed protein purified through immobilized metal affinity chromatography showed molecular mass of 60 kDa on SDS-PAGE. Results of thin-layer chromatography using inulin as a substrate showed the enzyme to be an exo-type inulinase capable of producing only monomeric fructose as a product. K_m and k_{cat} for the hydrolyses of inulin and sucrose were 2.28±0.08 mM and 358.05±20.38 min⁻¹, and 22.02±0.41 mM and 4619.11±215.12 min⁻¹, respectively. Optimal activity of the exoinulinase occurred at pH 7.0 and 50°C.

12/7/24

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17926033 BIOSIS NO.: 200400296790

Purification and characterization of an exoinulinase from *Aspergillus fumigatus*

AUTHOR: Gill Prabhjot Kaur; Manhas Rajesh Kumari; Singh Jatinder; Singh Prabhjeet (Reprint)

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JOURNAL: Applied Biochemistry and Biotechnology 117 (1): p19-32 April 2004

MEDIUM: print

ISSN: 0273-2289 (ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: An extracellular exoinulinase was purified from the crude extract of *Aspergillus fumigatus* by ammonium sulfate precipitation, followed by successive chromatographies on DEAE-Sephacel, Sephacryl S-200, concanavalin A-linked amino-activated silica, and Sepharose 6B columns. The enzyme was purified 25-fold, and the specific activity of the purified enzyme was 171 IU/mg of protein. Gel filtration chromatography revealed a molecular weight of about 200 kDa, and native polyacrylamide gel electrophoresis (PAGE) showed an electrophoretic mobility corresponding to a molecular weight of about 176.5 kDa. Sodium dodecyl sulfate-PAGE analysis revealed three closely moving bands of about 66, 62.7, and 59.4 kDa, thus indicating the heterotrimeric nature of this enzyme. The purified enzyme appeared as a single band on isoelectric focusing, with a pI of about 8.8. The enzyme activity was maximum at pH 5.5 and was stable over a pH range of 4.0-9.5, and the optimum temperature for enzyme activity was 60°C. The purified enzyme retained 35.9 and 25.8% activities after 4 h at 50 and 55°C, respectively. The inulin hydrolysis activity was

completely abolished with 1 mM Hg⁺⁺, whereas EDTA inhibited about 63% activity. As compared to sucrose, stachyose, and raffinose, the ~~purified~~ enzyme had lower K_m (0.25 mM) and higher V_{max} (333.3 IU/mg) values for ~~inulin~~.

12/7/25

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17878827 BIOSIS NO.: 200400247774

Biochemical and molecular characterization of a levansucrase from *Lactobacillus reuteri*.

AUTHOR: van Hijum S A F T; Szalowska E; van der Maarel M J E C; Dijkhuizen L (Reprint)

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JOURNAL: Microbiology (Reading) 150 (3): p621-630 March 2004 2004

MEDIUM: print

ISSN: 1350-0872 _(ISSN print)

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: *Lactobacillus reuteri* strain 121 employs a fructosyltransferase (FTF) to synthesize a fructose polymer (a fructan of the levan type, with beta(2fwdarw6) linkages) from sucrose or raffinose. ~~Purification~~ of this FTF (a levansucrase), and identification of peptide amino acid sequences, allowed isolation of the first *Lactobacillus levansucrase* gene (*lev*), encoding a ~~protein~~ (*Lev*) consisting of 804 amino acids. *Lev* showed highest similarity with an inulosucrase of *L. reuteri* 121 (*Inu*; producing an ~~inulin~~ polymer with beta(2fwdarw1)-linked fructosyl units) and with FTFs from streptococci. Expression of *lev* in *Escherichia coli* resulted in an active FTF (*LevDELTA773His*) that produced the same levan polymer (with only 2-3% beta(2fwdarw1fwdarw6) branching points) as *L. reuteri* 121 cells grown on raffinose. The low degree of branching of the *L. reuteri* levan is very different from bacterial levans known up to now, such as that of *Streptococcus salivarius*, having up to 30% branches. Although *Lev* is unusual in showing a higher hydrolysis than transferase activity, significant amounts of levan polymer are produced both in vivo and in vitro. *Lev* is strongly dependent on Ca²⁺ ions for activity. Unique properties of *L. reuteri* *Lev* together with *Inu* are: (i) the presence of a C-terminal cell-wall-anchoring motif causing similar expression problems in *Escherichia coli*, (ii) a relatively high optimum temperature for activity for FTF enzymes, and (iii) at 50degreeC, kinetics that are best described by the Hill equation.

12/7/26

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17729250 BIOSIS NO.: 200400100007

A porcine astrocyte/endothelial cell co-culture model of the blood-brain barrier.

AUTHOR: Jeliaskova-Mecheva Valentina V; Bobilya Dennis J (Reprint)

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JOURNAL: Brain Research Protocols 12 (2): p91-98 October 2003 2003
MEDIUM: print
ISSN: 1385-299X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A method for the isolation of porcine astrocytes as a simple extension of a previously described procedure for isolation of brain capillary endothelial cells from adolescent pigs (Methods Cell Sci. 17 (1995) 2) is described. The obtained astroglial culture ***purified*** through two passages and by the method of the selective detachment was validated by a phase contrast microscopy and through an immunofluorescent assay for the glial fibrillary acidic ***protein*** (GFAP). Porcine astrocytes were co-cultivated with porcine brain capillary endothelial cells (PBCEC) for the development of an in vitro blood-brain barrier (BBB) model. The model was visualized by an electron microscopy and showed elevated transendothelial electrical resistance and reduced ***inulin*** permeability. To our knowledge, this is the first report for the establishment of a porcine astrocyte/endothelial cell co-culture BBB model, which avoids interspecies and age differences between the two cell types, usually encountered in the other reported co-culture BBB models. Considering the availability of the porcine brain tissue and the close physiological and anatomical relation between the human and pig brain, the porcine astrocyte/endothelial cell co-culture system can serve as a reliable and easily reproducible model for different in vitro BBB studies.

12/7/27

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17526642 BIOSIS NO.: 200300480597

A novel enzyme of Bacillus sp. 217C-11 that produces ***inulin*** from sucrose.

AUTHOR: Wada Tadashi (Reprint); Ohguchi Masao; Iwai Yoshio
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JOURNAL: Bioscience Biotechnology and Biochemistry 67 (6): p1327-1334 June 2003 2003
MEDIUM: print
ISSN: 0916-8451
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We found a bacterium that converts sucrose to a useful material, using about 6,000 samples of bacteria isolated from soil. This bacterium, Bacillus sp. 217C-11, was identified according to Bergey's manual, and produced a highly efficient enzyme that converted sucrose into ***inulin***. So, the enzyme was ***purified*** to homogeneity through five chromatographic steps, to identify its enzymatic properties. The

molecular mass of the enzyme was estimated to be 45,000, and this enzyme was a monomer **protein** (by SDS-PAGE). The optimum pH and temperature of this enzyme were 7-8 and 45-50degreeC, respectively. The enzyme reacted only with sucrose, but did not with other disaccharides, fructooligosaccharides and **inulin**. This paper will show that our enzyme is a novel one, which is different from the other well-known enzymes concerned in **inulin** production.

12/7/28

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17300256 BIOSIS NO.: 200300258900

Porcine co-culture model of the blood-brain barrier.

AUTHOR: Jeliaskova-Mecheva Valentina Vassileva (Reprint); Gauthier Nicole; Hinman Elizabeth; Bobilya Dennis J

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JOURNAL: FASEB Journal 17 (4-5): pAbstract No. 79.2 March 2003 2003

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CONFERENCE/MEETING: FASEB Meeting on Experimental Biology: Translating the Genome San Diego, CA, USA April 11-15, 2003; 20030411

SPONSOR: FASEB

ISSN: 0892-6638 _(ISSN print)

DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Astrocytes play an important role in supporting blood-brain barrier (BBB) functions. We developed a method for isolation of porcine astrocytes as an adjunct procedure for isolation of porcine brain capillary endothelial cells (BCEC) previously developed in our laboratory. After physical and enzymatic digestion of the porcine brain tissue, followed by removal of most lipophilic matter, the tissue suspension was passed through screens with decreasing pore sizes. Cells that filtered through a 20 mum screen were collected and seeded in a Minimum Essential Medium with 10% Fetal Bovine Serum. By selective detachment and reattachment during passaging, we obtained a highly **purified** population of cells which tested positive for the Glial Fibrillary Acidic **Protein**. In this way we isolated BCEC and astrocytes using only one procedure. Both cell types were used in a BBB co-culture model, which avoids interspecies and age differences between the two cell types, usually encountered in the other BBB co-culture models. Transendothelial electrical resistance increased ($P < 0.0001$) and **inulin** passage was reduced ($P < 0.001$) in the co-culture model in comparison with the BCEC monolayer BBB model. This porcine co-culture model established by a procedure for simultaneous isolation of astrocytes and BCEC can serve as a reliable model for studies of the BBB.

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17211600 BIOSIS NO.: 200300170319

Cloning and characterization of cycloinulooligosaccharide

fructanotransferase (CFTase) from *Bacillus polymyxa* MGL21.

AUTHOR: Jeon Sung-Jong; You Dong-Ju; Kwon Hyun-Ju; Kanaya Shigenori;
Kunihiro Namio; Kim Kwang-Hyeon; Kim Young-Hee; Kim Byung-Woo (Reprint)

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JOURNAL: Journal of Microbiology and Biotechnology 12 (6): p921-928
December 2002 2002

MEDIUM: print

ISSN: 1017-7825

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Microorganism producing extracellular CFTase was isolated from soil and designated as *Bacillus polymyxa* MGL21. The gene encoding the CFTase (cft) from *B. polymyxa* MGL21 was cloned and sequenced. The ORF of the cft gene was composed of 3,999 nucleotides, encoding a %protein% (1,333 amino acids) with a predicted molecular mass of 149,375 Da. Sequence analysis indicated that CFTase was divided into five distinct regions. CFTase contained three regions of repeat sequences at the N-terminus and C-terminus. The endo-inulinase region of homology (ERH) of CFTase was similar to that of *Pseudomonas mucidolens* endo-inulinase (50% identity, 259 amino acids). Furthermore, CFTase possessed a highly conserved core region, which is considered to be functional for the hydrolysis reaction of %inulin%. The cft gene was expressed in a His-tagged form in *Escherichia coli* cells, and the His-tagged CFTase was %purified% to homogeneity. The optimal temperature and pH for CFTase activity were found to be 50degreeC and 9.0, respectively. The enzyme activity was completely inhibited by 10 mM Ag⁺ and Cu²⁺. Thin-layer chromatography analyses indicated that CFTase catalyzed not only the cyclization reaction but also disproportionation and hydrolysis reactions as well.

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17201585 BIOSIS NO.: 200300160304

Cloning and characterization of an exoinulinase from *Bacillus polymyxa*.

AUTHOR: Kwon Hyun-Ju; Jeon Sung-Jong; You Dong-Ju; Kim Kwang-Hyeon; Jeong
Yong-Kee; Kim Young-Hee; Kim Young-Man; Kim Byung-Woo (Reprint)

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JOURNAL: Biotechnology Letters 25 (2): p155-159 January 2003 2003

MEDIUM: print

ISSN: 0141-5492

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A gene encoding an exoinulinase (inu) from *Bacillus polymyxa* MGL21 was cloned and sequenced. It is composed of 1455 nucleotides,

encoding a protein (485 amino acids) with a molecular mass of 55 522 Da. Inu was expressed in Escherichia coli and the His-tagged exoinulinase was purified. The purified enzyme hydrolyzed sucrose, levan and raffinose, in addition to inulin, with a sucrose/inulin ratio of 2. Inulinase activity was optimal at 35°C and pH 7, was completely inactivated by 1 mM Ag⁺ or Hg²⁺. The K_m and V_{max} values for inulin hydrolysis were 0.7 mM and 2500 μM min⁻¹ mg⁻¹ protein. The enzyme acted on inulin via an exo-attack to produce fructose mainly.

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17108624 BIOSIS NO.: 200300067343

Purification and properties of an extracellular exoinulinase from *Penicillium* sp. strain TN-88 and sequence analysis of the encoding gene.

AUTHOR: Moriyama Satoshi; Akimoto Hidetoshi; Suetsugu Norio; Kawasaki Soushi; Nakamura Toyohiko; Ohta Kazuyoshi (Reprint)

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JOURNAL: Bioscience Biotechnology and Biochemistry 66 (9): p1887-1896

September 2002 2002

MEDIUM: print

ISSN: 0916-8451

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: An exoinulinase, P-I, was purified from the culture filtrate of *Penicillium* sp. strain TN-88 grown on inulin. The enzyme was homogeneous as judged by SDS-polyacrylamide gel electrophoresis with an apparent Mr of 81 kDa. The purified enzyme had extremely high specific activity, 743 U/mg, toward inulin. Inulinase activity was optimal at pH 4.0 and 55°C. A genomic DNA and cDNAs encoding this protein were cloned and sequenced. The exoinulinase gene (*inuD*) was present as a single copy in the genome. An open reading frame of 2,106 bp was interrupted by a single intron of 56 bp, and encoded a 25-amino acid signal peptide and a 677-amino acid mature protein. The mature protein contained two Cys residues and eight potential N-linked glycosylation sites. The 5'-noncoding region had a putative CAAT box at position -239. Four distinct transcription start points were observed at positions -98 (A), -91 (A), -80 (A), and -76 (A) from the start codon. The exoinulinase gene *inuD* was located 860-bp upstream of the previously reported endoinulinase gene *inuC* in the opposite direction of transcription.

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17102056 BIOSIS NO.: 200300060775

Characterization of a purified beta-fructofuranosidase from

Bifidobacterium infantis ATCC 15697.
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JOURNAL: Letters in Applied Microbiology 35 (6): p462-467 2002 2002
MEDIUM: print
ISSN: 0266-8254
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Aims: To characterize the beta-fructofuranosidase of
Bifidobacterium infantis ATCC 15697 and to compare it with other
bacterial beta-fructofuranosidases. Methods and Results: The
beta-fructofuranosidase of B. infantis ATCC 15697 was %%%purified%%% 46.8
times over the crude extract by anion exchange chromatography,
ultrafiltration and gel filtration. The sequence of 15 amino acid
residues of the NH2 terminal was determined. This enzyme was a monomeric
%%%protein%%% (Mr 70 kDa) with beta-fructofuranosidase and invertase
activities. The isoelectric point was pH 4.3, the optimum pH 6.0 and pKas
(4.5 and 7.2) of two active groups were obtained. The activities were
inhibited by Hg2+ and p-chloromercuribenzoic acid (pCMB). The optimal
temperature was 37degreeC and activities were unstable at 55degreeC.
beta-fructofuranosidase activity was more efficient than that of
invertase with Vm/Km ratios of 0.65 and 0.025X10-3 1 min-1 mg-1,
respectively. The enzyme catalyses the hydrolysis of
fructo-oligosaccharides, sucrose and %%%inulin%%% at relative velocities
of 100, 10 and 6, respectively. Conclusions: The enzyme of B. infantis
ATCC 15697 is an exo-inulinase which has beta-fructofuranosidase and
invertase activities. This %%%protein%%% was different from the
beta-fructofuranosidase of another strain of B. infantis (B. infantis JCM
no. 7007). Significance and Impact of the Study: A better knowledge of
bacterial beta-fructofuranosidases, especially from bifidobacteria, has
been gained.

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17042069 BIOSIS NO.: 200300000788
Isolation of a new heterodimeric lectin with mitogenic activity from
fruiting bodies of the mushroom Agrocybe cylindracea.
AUTHOR: Wang Hexiang; Ng T B (Reprint); Liu Qinghong
AUTHOR ADDRESS: Department of Biochemistry, Chinese University of Hong
Kong, Shatin, New Territories, Hong Kong, China**China
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JOURNAL: Life Sciences 70 (8): p877-885 January 11, 2002 2002
MEDIUM: print
ISSN: 0024-3205 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: From the dried fruiting bodies of the mushroom Agrocybe

cylindracea a heterodimeric lectin with a molecular weight of 31.5 kDa and displaying high hemagglutinating activity was isolated. The molecular weights of its subunits were 16.1 kDa and 15.3 kDa respectively. The larger and the smaller subunits resembled Agaricus bisporus lectin and fungal immunomodulatory **protein** from Volvariella volvacea respectively in N-terminal sequence. The lectin was adsorbed on DEAE-cellulose in 10 mM Tris-HCl buffer (pH 7.4) and was eluted by the same buffer containing 150 mM NaCl. It was adsorbed on SP-Sepharose in 10mM NH₄OAc (pH 4.5) and eluted by approximately 0.19 M NaCl in the same buffer. The lectin was obtained in a **purified** form after the mushroom extract had been subjected to (NH₄)₂SO₄ precipitation and the two aforementioned ion exchange chromatographic steps. The lectin exhibited potent mitogenic activity toward mouse splenocytes. The hemagglutinating activity of the lectin was inhibited by lactose, sialic acid and **inulin**.

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16931881 BIOSIS NO.: 200200525392

Characterization of a novel fructosyltransferase from Lactobacillus reuteri that synthesizes high-molecular-weight **inulin** and **inulin** oligosaccharides

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JOURNAL: Applied and Environmental Microbiology 68 (9): p4390-4398
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RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Fructosyltransferase (FTF) enzymes produce fructose polymers (fructans) from sucrose. Here, we report the isolation and characterization of an FTF-encoding gene from Lactobacillus reuteri strain 121. A C-terminally truncated version of the ftf gene was successfully expressed in Escherichia coli. When incubated with sucrose, the **purified** recombinant FTF enzyme produced large amounts of fructo-oligosaccharides (FOS) with beta-(2fwdarw1)-linked fructosyl units, plus a high-molecular-weight fructan polymer (>107) with beta-(2fwdarw1) linkages (an **inulin**). FOS, but not **inulin**, was found in supernatants of L. reuteri strain 121 cultures grown on medium containing sucrose. Bacterial **inulin** production has been reported for only Streptococcus mutans strains. FOS production has been reported for a few bacterial strains. This paper reports the first-time isolation and molecular characterization of (i) a Lactobacillus ftf gene, (ii) an inulosucrase associated with a generally regarded as safe bacterium, (iii) an FTF enzyme synthesizing both a high molecular weight **inulin** and FOS, and (iv) an FTF **protein** containing a cell wall-anchoring LPXTG motif. The biological relevance and potential health benefits of an inulosucrase associated with an L. reuteri strain remain to be established.

12/7/35

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16865504 BIOSIS NO.: 200200459015

Cloning and characterization of a levanbiohydrolase from *Microbacterium laevaniformans* ATCC 15953

AUTHOR: Song Eun-Kyung; Kim Hyunjin; Sung Hee-Kyung; Cha Jaeho (Reprint)

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JOURNAL: Gene (Amsterdam) 291 (1-2): p45-55 29 May, 2002 2002

MEDIUM: print

ISSN: 0378-1119

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LANGUAGE: English

ABSTRACT: An extracellular levanbiohydrolase gene, *levM*, from *Microbacterium laevaniformans* ATCC 15953 was cloned and its nucleotide sequence was determined. Nucleotide sequence analysis of this gene revealed a 1863 bp open reading frame coding for a protein of 621 amino acids. The deduced amino acid sequence of the *levM* gene exhibited 28-47% sequence identities with levanases, levanfructotransferases, and inulinases. The *LevM* was overexpressed by using a T7 promoter in *Escherichia coli* BL21 (DE3) and purified 24-fold from culture supernatant. The molecular weight of this enzyme was 68,800 Da based on the sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The optimum pH and temperature of this enzyme for levan degradation was pH 6.0 and 30°C, respectively. Thin-layer and high-performance liquid chromatography analyses proved that the enzyme produced mostly levanbiose from levan in an exo-acting manner. The recombinant enzyme also hydrolyzed inulin, 1-kestose, and nystose, indicating that the enzyme cleaves not only beta-2,6-linkage of levan but also beta-2,1-linkage of fructooligosaccharides. This is the first report on a gene encoding a levanbiohydrolase that produces levanbiose as a major degradation product.

12/7/36

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16859990 BIOSIS NO.: 200200453501

Purification and properties of a heat stable inulin

fructotransferase (DFA III-producing) from *Arthrobacter pascens* T13-2

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JOURNAL: Carbohydrate Polymers 50 (2): p117-121 1 November, 2002 2002

MEDIUM: print

ISSN: 0144-8617

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RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: An *inulin* fructotransferase (DFA III-producing) (EC 2.4.1.93) from *Arthrobacter pascens* T13-2 was purified and the properties of the enzyme were investigated. The enzyme was purified from a culture supernatant of the microorganism 18.5-fold with a yield of 13.1% by Super Q Toyopearl chromatographies and a butyl Toyopearl chromatography. It gave a single band on SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The molecular mass of the enzyme was estimated to be 44 000 by SDS-PAGE and 79 000 by gel filtration and was therefore considered to be a dimer. The N-terminal amino acid sequence was determined as Ala-Gln-Asp-Ala-Lys-Ala-Gly-Pro-Phe-Asn-Ser-Pro-Asn-Thr-Tyr-Asp-Val-Thr. The enzyme showed maximum activity at pH 5.5-6.0. The optimum temperature for the enzyme activity was 50°C. The enzyme was stable up to 75°C. The enzyme activity was inhibited strongly by Hg²⁺, and inhibited slightly by Fe³⁺, and Zn²⁺. An immobilized enzyme was prepared using Chitopearl BCW3510 as a carrier. The immobilized enzyme was able to use eight times without a significant loss of the enzyme activity.

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16605680 BIOSIS NO.: 200200199191

Purification, characterization, gene cloning and preliminary X-ray data of the *exo-inulinase* from *Aspergillus awamori*

AUTHOR: Arand Michael; Golubev Alexander M; Neto J R Brandao; Polikarpov Igor; Wattiez R; Korneeva Olga S; Eneyskaya Elena V; Kulminskaya Anna A; Shabalin Konstantin A; Shishliannikov Sergei M; Chepurnaya Olga V; Neustroev Kirill N (Reprint)

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JOURNAL: Biochemical Journal 362 (1): p131-135 15 February, 2002 2002

MEDIUM: print

ISSN: 0264-6021

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LANGUAGE: English

ABSTRACT: Extracellular *exo-inulinase* has been isolated from a solid-phase culture of the filamentous fungus *Aspergillus awamori* var. 2250. The apparent molecular mass of the monomer enzyme was 69 ± 1 kDa, with a pI of 4.4 and a pH optimum of 4.5. The enzyme hydrolysed the β -(2 → 1)-fructan (*inulin*) and β -(2 → 6)-fructan (levan) via *exo*-cleavage, releasing fructose. The values for the Michaelis constants K_m and V_{max} in the hydrolysis of *inulin* were 0.003 ± 0.0001 mM and 175 ± 5 μ mol/min per mg and 1.2 ± 0.02 μ mol/min per mg, respectively. The gene and cDNA encoding the *A. awamori* *exo-inulinase* were cloned and sequenced. The amino acid sequence indicated that the *protein* belongs to glycoside hydrolase family 32. A surprisingly high similarity was found to fructosyltransferase from *Aspergillus foetidus* (90.7% on the level of the amino acid sequence), despite the fact that the latter enzyme is unable to hydrolyse *inulin* and levan. Crystals of the native *exo-inulinase* were obtained and found to belong to the orthorhombic space group P212121 with cell parameters a =

64.726 ANG (1ANG = 0.1 nm), b = 82.041 ANG and c = 136.075 ANG. Crystals diffracted beyond 1.54 ANG, and useful X-ray data were collected to a resolution of 1.73 ANG.

12/7/38

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15979362 BIOSIS NO.: 200100151201

Molecular characterization of cycloinulooligosaccharide fructanotransferase from *Bacillus macerans*

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JOURNAL: Applied and Environmental Microbiology 67 (2): p995-1000
February, 2001 2001

MEDIUM: print

ISSN: 0099-2240

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Cycloinulooligosaccharide fructanotransferase (CFTase) converts *inulin* into cyclooligosaccharides of beta-(2fwdarw1)-linked D-fructofuranose by catalyzing an intramolecular transfructosylation reaction. The CFTase gene was cloned and characterized from *Bacillus macerans* CFC1. The CFTase gene encoded a polypeptide of 1,333 amino acids with a calculated Mr of 149,563. Western blot and zymography analyses revealed that the CFTase with a molecular mass of 150 kDa (CFT150) was processed (between Ser389 and Phe390 residue) to form a 107-kDa *protein* (CFT107) in the *B. macerans* CFC1 cells. The processed CFT107 was similar in its mass to the previously *purified* CFTase from *B. macerans* CFC1. The CFT107 enzyme was produced by *B. macerans* CFC1 but was not detected from the recombinant *Escherichia coli* cells, indicating that the processing event occurred in a host-specific manner. The two CFTases (CFT150 and CFT107) exhibited the same enzymatic properties, such as influences of pH and temperature on the enzyme activity, the intermolecular transfructosylation ability, and the ability of hydrolysis of cycloinulooligosaccharides produced by the cyclization reaction. However, the thermal stability of CFT107 was slightly higher than that of CFT150. The most striking difference between the two enzymes was observed in their Km values; the value for CFT150 (1.56 mM) was threefold lower than that for CFT107 (4.76 mM). Thus, the specificity constant (kcat/Km) of CFT150 was about fourfold higher than that of CFT107. These results indicated that the N-terminal 358-residue region of CFT150 played a role in increasing the enzyme's binding affinity to the *inulin* substrate.

12/7/39

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15930433 BIOSIS NO.: 200100102272

Gene cloning and functional characterization by heterologous expression of the fructosyltransferase of *Aspergillus sydowi* IAM 2544

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JOURNAL: Applied and Environmental Microbiology 67 (1): p363-370 January,
2001 2001
MEDIUM: print
ISSN: 0099-2240
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have **purified** a fructosyltransferase from conidia of the
inulin-producing fungus *Aspergillus sydowi* IAM 2544 and obtained
peptide sequences from proteolytic fragments of the **protein**. With
degenerated primers, we amplified a PCR fragment that was used to screen
a cDNA library. The fructosyltransferase gene from *Aspergillus sydowi*
(EMBL accession no. AJ289046) is expressed in conidia, while no
expression could be detected in mycelia by Northern blot analysis of
mycelial RNA. The gene encodes a **protein** with a calculated
molecular mass of 75 kDa that is different from all fructosyltransferases
in the databases. The only homology that could be detected was to the
invertase of *Aspergillus niger* (EMBL accession no. L06844). The gene was
functionally expressed in *Escherichia coli*, yeast, and potato plants.
With **protein** extracts from transgenic bacteria and yeast,
fructooligosaccharides could be produced in vitro. In transgenic potato
plants, **inulin** molecules of up to 40 hexose units were synthesized
in vivo. While in vitro experiments with **protein** extracts from
conidia of *Aspergillus sydowi* yielded the same pattern of
oligosaccharides as extracts from transformed bacteria and yeast, in vivo
inulin synthesis with fungal conidia leads to the production of a
high-molecular-weight polymer.

12/7/40

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15772036 BIOSIS NO.: 200000490349
Production, **purification** and characterization of an extracellular
inulinase from *Kluyveromyces marxianus* var. *bulgaricus*
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JOURNAL: Journal of Industrial Microbiology and Biotechnology 25 (2): p
63-69 August, 2000 2000
MEDIUM: print
ISSN: 1367-5435
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The yeast *Kluyveromyces marxianus* var. *bulgaricus* produced large
amounts of extracellular inulinase activity when grown on **inulin**,
sucrose, fructose and glucose as carbon source. This **protein** has
been **purified** to homogeneity by using successive DEAE-Trisacryl
Plus and Superose 6HR 10/30 columns. The **purified** enzyme showed a

relative molecular weight of 57 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 77 kDa by gel filtration in Superose 6 HR 10/30. Analysis by SDS-PAGE showed a unique polypeptide band with Coomassie Blue stain and nondenaturing PAGE of the purified enzyme obtained from media with different carbon sources showed the band, too, when stained for glucose oxidase activity. The optimal hydrolysis temperature for sucrose, raffinose and inulin was 55°C and the optimal pH for sucrose was 4.75. The apparent K_m values for sucrose, raffinose and inulin are 4.58, 7.41 and 86.9 mg/ml, respectively. Thin layer chromatography showed that inulinase from *K. marxianus* var. *bulgaricus* was capable of hydrolyzing different substrates (sucrose, raffinose and inulin), releasing monosaccharides and oligosaccharides. The results obtained suggest the hypothesis that enzyme production was constitutive.

12/7/41

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15642218 BIOSIS NO.: 200000360531

Cloning and characterization of *Pseudomonas mucidolens* exoinulinase

AUTHOR: Young-Man Kwon; Kim HWA-Young; Choi Yong-Jin (Reprint)

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JOURNAL: Journal of Microbiology and Biotechnology 10 (2): p238-243 April, 2000 2000

MEDIUM: print

ISSN: 1017-7825

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: An exoinulinase (beta-D-fructofuranosidase) gene was cloned by chromosome walking along the upstream region of the endoinulinase gene of *Pseudomonas mucidolens* isolated from soil. The exoinulinase gene consisted of an ORF of 1,506 bp encoding a polypeptide of 501 amino acids with a deduced molecular weight of 55,000. The exoinulinase produced by the recombinant *Escherichia coli* DH5alpha strain was also purified to homogeneity as determined by SDS-PAGE and a zymogram. The molecular weight of the purified exoinulinase according to both SDS-PAGE and gel filtration matched the deduced molecular weight of the protein described above, thereby indicating that the native form of the exoinulinase was a monomer. The purified enzyme hydrolyzed sucrose, raffinose, levan, in addition to inulin, with an S/I activity value of 2.0. Furthermore, no inulo-oligomers were liberated from the inulin substrate in the enzymatic reaction mixtures incubated for 90 min at 55°C. Taken together, these results indicate that the purified beta-D-fructofuranosidase was an exoinulinase. The pH and temperature optima of the exoinulinase were pH 6.0 and 55°C, respectively. The enzyme had no apparent requirement for a cofactor, and its activity was completely inactivated by Ag⁺, Hg²⁺, and Zn²⁺. Kinetic experiments gave K_m , V_{max} , and K_{cat} values for inulin of 11.5 mM, 18 nM/s, and 72 s⁻¹, respectively. The exoinulinase was fairly stable in broad pH conditions (pH 3-9), and at pH 6.0 it showed a residual activity of about 70% after 4 h incubation at 55°C.

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15484886 BIOSIS NO.: 200000203199

An efficient %%%purification%%% with a high recovery of the %%%inulin%%% fructotransferase of *Arthrobacter* sp. A-6 from recombinant *Escherichia coli*

AUTHOR: Kim Hwa-Young; Kim Il-Hwan; Choi Yong-Jin (Reprint)

AUTHOR ADDRESS: Graduate School of Biotechnology, Korea University, Seoul, 136-701, South Korea**South Korea

JOURNAL: Biotechnology Letters 22 (4): p291-293 Feb., 2000 2000

MEDIUM: print

ISSN: 0141-5492

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: %%%Inulin%%% fructotransferase (IFTase, EC 2.4.1.93) of *Arthrobacter* sp. A-6 was %%%purified%%% from a cell extract of the recombinant *Escherichia coli* DH5 alpha/pDFE cells carrying the IFTase gene using heat treatment followed by gel filtration. The enzyme was %%%purified%%% 45-fold to apparent homogeneity with a recovery of 79%. SDS-PAGE yielded a single %%%protein%%% band of Mr 46.5 kDa. The recombinant IFTase had a similar thermostability as the original enzyme from *Arthrobacter* sp. A-6.

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15377446 BIOSIS NO.: 200000095759

Production of inulooligosaccharides from %%%inulin%%% by a novel endoinulinase from *Xanthomonas* sp

AUTHOR: Park J P; Bae J T; You D J; Kim B W; Yun J W (Reprint)

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JOURNAL: Biotechnology Letters 21 (12): p1043-1046 Dec., 1999 1999

MEDIUM: print

ISSN: 0141-5492

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A novel inulinolytic microorganism, *Xanthomonas* sp. produced an endoinulinase, to be used for inulooligosaccharide (IOS) formation from %%%inulin%%%, at an activity of 11 units ml⁻¹ (1.2 mg %%%protein%%% ml⁻¹). The endoinulinase was optimally active at 45degreeC and pH 6.0. Batchwise production of IOS was carried out by the partially %%%purified%%% endoinulinase with a maximum yield of about 86% on a total sugar basis with 10 g %%%inulin%%% l⁻¹. The major IOS components were DP (degree of polymerization) 5 and 6 with trace amount of smaller oligosaccharides.

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15107857 BIOSIS NO.: 199900367517

Production and characterization of raffinose-hydrolysing and invertase activities of *Aspergillus fumigatus*

AUTHOR: de Rezende S T (Reprint); Felix C R

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JOURNAL: Folia Microbiologica 44 (2): p191-195 1999 1999

MEDIUM: print

ISSN: 0015-5632

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Raffinose-type galactose oligosaccharides constitute a substantial part (40 %) of the soluble sugars present in soybean seeds and are responsible for flatulence following ingestion of soybean and other legumes. Enzymic hydrolysis of these oligosaccharides would improve the nutritional value of soybean milk. *Aspergillus fumigatus* produces substantial raffinose-hydrolysing and invertase activities when grown on wheat straw. Three proteins displaying maximal activity at pH 4.5-5.5 and 55-60 degreeC and having molar mass of 66.8, 50.3 and 30.2 kDa were %purified%. Raffinose and sucrose were hydrolyzed with equivalent affinities by each %protein%. Nevertheless, the Km and Vlim values determined for hydrolysis of sucrose by the 66.8 kDa enzyme differed from those determined with the 50.3 kDa %protein%. Glucose was produced when sucrose was the substrate. The three proteins hydrolyzed also stachyose but not melibiose, maltose, %inulin% or 4-nitrophenyl alpha-D-galactopyranoside. *A. fumigatus* enzymes may be candidates for processing of soybean milk to reduce its flatulence potential.

12/7/45

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15096099 BIOSIS NO.: 199900355759

%Purification% and properties of a second fructan exohydrolase from the roots of *Cichorium intybus*

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JOURNAL: Physiologia Plantarum 106 (1): p28-34 May, 1999 1999

MEDIUM: print

ISSN: 0031-9317

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A 1-FEH II (1-fructan exohydrolase, EC 3.2.1.80) was %purified% from forced chicory roots (*Cichorium intybus* L. var. foliosum cv. Flash) by a combination of ammonium sulfate precipitation,

concanavalin A (Con A) affinity chromatography and anion and cation exchange chromatography. This protocol produced a 70-fold purification and a specific activity of 52 nkat mg⁻¹ protein. The apparent size of the enzyme was 60 kDa as estimated by gel filtration and 64 kDa on SDS-PAGE. Optimal activity was found between pH 5.0 and 5.5. The temperature optimum was around 35°C. No product other than fructose could be detected with inulin as the substrate. The purified enzyme exhibited hyperbolic saturation kinetics with an apparent K_m of 58 mM for 1-kestose (Kes) and 64 mM for 1,1-nystose (Nys). The purified 1-FEH II hydrolyzed the beta(2 foward 1) linkages in inulin, Kes and Nys at rates at least 5 times faster than the beta(2 foward 6) linkages in levan oligosaccharides and levanbiose. Fructose did not affect the 1-FEH II activity but sucrose (Suc) was a strong inhibitor of this 1-FEH II (K_i = 5.9 mM). The enzyme was partially inhibited by Na-EDTA and CaCl₂ (1 mM).

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14976556 BIOSIS NO.: 199900236216

Purification and characterization of *Aspergillus ficuum* endoinulinase
AUTHOR: Uhm Tai-Boong (Reprint); Chung Mi Sun; Lee Sun Hee; Gourronc
Francoise; Housen Isabelle; Kim Jong Hwa; Van Beeumen Josef; Haye Bernard
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JOURNAL: Bioscience Biotechnology and Biochemistry 63 (1): p146-151 Jan.,
1999 1999

MEDIUM: print

ISSN: 0916-8451

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RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Endoinulinase from *Aspergillus ficuum*, which catalyzes the hydrolysis of inulin via an endo-cleavage mode, was purified by chromatography from Novozym 230 as a starting commercial enzyme mixture on CM-Sephadex and DEAE-Sephadex, and by preparative electrophoresis under native conditions. The enzyme was estimated to be pure on the basis of its I/S ratio, whose value was infinite in our assay conditions. Two forms separated by using this method. SDS gel electrophoresis showed the two purified forms to respectively exhibit molecular weights of 64,000 ± 500 and 66,000 ± 1,000. The results of deglycosylation indicated that the two forms were originally the same protein but with different sugar contents. A molecular weight of 54,800 ± 1,500 was found by gel filtration of the native enzyme, indicating the native functional protein to be a monomer. The enzyme showed nearly absolute substrate specificity towards inulin and inulooligosaccharides, and acted via an endo-attack to produce mainly inulotriose during the late stage of the reaction. The apparent K_m and V_{max} values for inulin hydrolysis were 8.1 ± 1.0 mM and 773 ± 60 U/mg, respectively. The internal peptides of the enzyme showed sequence homology to the endoinulinase of *Penicillium purpurogenum*.

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14948672 BIOSIS NO.: 199900208332

Structure of the enzymatically synthesized fructan %inulin%

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JOURNAL: Carbohydrate Research 313 (3-4): p165-174 Dec. 15, 1998 1998

MEDIUM: print

ISSN: 0008-6215

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Construction, %purification% and characterization of a fusion %protein% of maltose-binding %protein% of Escherichia coli and the fructosyltransferase of Streptococcus mutans is described. With the %purified% %protein%, in vitro synthesis of %inulin% was performed. The obtained polysaccharide was characterized by high-performance size-exclusion chromatography (HPSEC) and static light scattering (SLS) in dilute aqueous and dimethyl sulfoxide solution. For all samples very high molecular weights between 60 X 10⁶ and 90 X 10⁶ g/mol and a remarkable small polydispersity index of 1.1 have been determined. Small root-mean-square radii of gyration point to a compact conformation in dilute solution. No difference between native and enzymatically synthesized %inulin% was observed by X-ray powder diffraction and thermoanalysis of solid samples.

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14757438 BIOSIS NO.: 199900017098

Characterization of a fructan exohydrolase %purified% from barley stems that hydrolyzes multiple fructofuranosidic linkages

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JOURNAL: Plant Physiology and Biochemistry (Paris) 36 (10): p715-720 Oct.,
1998 1998

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ISSN: 0981-9428

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Barley (Hordeum vulgare cv Morex) fructan exohydrolase (EC 3.2.1.80) was %purified% by precipitation with ammonium sulfate and chromatography on anion exchange and lectin affinity columns. The final enzyme preparation was homogenous as determined by the presence of a single band on silver stained SDS-PAGE and IEF gels. The %purified%

%%protein%% had a molecular mass of 33 kDa and a pI of 7.8. Analyses of relative hydrolytic rates of various fructans were determined by measuring released fructose by pulsed electrochemical detection after separation of reactions by HPLC. The %%purified%% enzyme hydrolyzed beta-2,1-linkages in 6G,1-kestotetraose, 1 and 6G-kestotetraose, 1,1-kestotetraose, and 1-kestotriose with relative rates of 100:96:85:88. This enzyme slowly hydrolyzed the beta-2,6-linkages in 6G-kestotriose and in 6G,6-kestotetraose and sucrose with relative rates of 5:4:3 compared to 6G, 1-kestotetraose hydrolysis rates arbitrarily set at 100. The substrate attack pattern, determined by identifying products from hydrolysis of %%purified%% fructan tetrasaccharides, was of the multichain type. Sucrose was a mixed-type inhibitor of %%inulin%% hydrolysis.

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14506977 BIOSIS NO.: 199800301224

Downstream processing of inulinase: Comparison of different techniques

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JOURNAL: Applied Biochemistry and Biotechnology 70-72 (0): p505-511

Spring, 1998 1998

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RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Candida kefyr DSM 70106 was cultivated in a medium containing %%inulin%% as a carbon source. About 92% of the inulinase was recovered directly from the medium. Different concentration (Cf) and enrichment (Ef) factors were obtained, using the following methods: Cross-flow filtration (microfiltration and cell diafiltration were carried out using a rotary filter; enzyme ultrafiltration and diafiltration were performed using a cassette module): Cf = 7.5 and Ef = 2.2; liquid-liquid extraction of N-Benzyl-N-Dodecyl-N-bis(2-hydroxyethyl) ammonium chloride (BDBAC) reversed micelles: Cf = 2.5 and Ef = 2.7; and expanded-bed adsorption: Cf = 2.8 and Ef = 4.3.

12/7/50

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14461162 BIOSIS NO.: 199800255409

ATP-dependent human erythrocyte glutathione-conjugate transporter. II.

Functional reconstitution of transport activity

AUTHOR: Awasthi Sanjay (Reprint); Singhal Sharad S; Pikula Slawomir; Piper

John T; Srivastava Sanjay K; Torman Robert T; Bandorowicz-Pikula Joanna;

Lin James T; Singh Shivendra V; Zimniak Piotr; Awasthi Yogesh C

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JOURNAL: Biochemistry 37 (15): p5239-5248 April 14, 1998 1998

MEDIUM: print
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Purified dinitrophenyl S-glutathione (DNP-SG) ATPase was reconstituted into artificial liposomes prepared from soybean asolectin. Electron micrography confirmed the formation of unilamellar vesicles with an average radius of 0.25 μm . Intravesicular volume estimated by incorporation of radiolabeled inulin into the vesicles was found to be $19.7 \pm 1.3 \mu\text{L/mL}$ reconstitution solution. Accumulation of the glutathione-conjugate of CDNB, DNP-SG, and of doxorubicin (DOX) in the proteoliposomes was increased in the presence of ATP as compared to equimolar ADP or adenosine 5'-(beta,gamma-methylene)triphosphate tetralithium. ATP-dependent transmembrane movement of DOX and DNP-SG into DNP-SG ATPase-reconstituted vesicles was saturable with respect to time, sensitive to the osmolarity of the assay medium, and temperature dependent. The energy of activation was found to be 12 and 15 kcal/mol for DNP-SG and DOX, respectively. Optimal temperature for transport was 37 degreeC. Saturable transport was demonstrated for DNP-SG (V_{max} of $433 \pm 20 \text{ nmol/min/mg}$ of protein, $K_{\text{mATP}} = 2.4 \pm 0.3 \text{ mM}$ and $K_{\text{mDNP-SG}} = 36 \pm 5 \mu\text{M}$) as well as DOX ($V_{\text{max}} = 194 \pm 19 \text{ nmol/min/mg}$ of protein, $K_{\text{mATP}} = 2.5 \pm 0.6 \text{ mM}$ and $K_{\text{mDOX}} = 2.4 \pm 0.7 \mu\text{M}$). The kinetic data for both DNP-SG and DOX transport were consistent with a random bi-bi sequential reaction mechanism. DOX was found to be a competitive inhibitor of DNP-SG transport with K_{i} of $1.2 \pm 0.2 \mu\text{M}$ and DNP-SG was found to be a competitive inhibitor of DOX transport with K_{i} of $13.3 \pm 2.6 \mu\text{M}$.

12/7/51
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14391894 BIOSIS NO.: 199800186141
Purification and properties of inulinase from Kluyveromyces sp. Y-85
AUTHOR: Wei Wenling; Yu Xiawen; Dai Ya; Zheng Jing; Xie Zhong
AUTHOR ADDRESS: Dep. Biol., Xiamen Univ., Xiamen 361005, China**China
JOURNAL: Weishengwu Xuebao 37 (6): p443-448 Dec., 1997 1997
MEDIUM: print
ISSN: 0001-6209
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: Chinese

ABSTRACT: The crude endocellular inulinase from Kluyveromyces sp. Y-85 was purified to two components, designated as EI and EII, using PEG6000-phosphate buffer extraction, $(\text{NH}_4)_2\text{SO}_4$ fractionation, DEAE chromatography and gel filtration (Protein-PAK); The crude exocellular inulinase from this strain was purified to Eexo by means of PEG6000-phosphate buffer extraction, double DEAE-Sephacel chromatography, Sephadex G-150 gel filtration. EI, EII and Eexo were demonstrated to be homogeneous by Waters 650E protein purification system. Their molecular weights are 42kD, 65kD and 57kD, respectively. All the inulinases were glycoproteins containing a saccharide (from 25% to 35%) and belonged to the endo-inulinase. In

addition, EI, EII, Eexo were optimally reactive at pH 4.6, 4.5, 4.6 and at 52 degree C, 52 degree C, 55 degree C, respectively. Ag⁺, Hg²⁺ and PCMB inhibited these enzymes' activity strongly. The products of raw inulin extracted from *Helianthus tuberosus* hydrolyzed by these three enzymes were fructose (86.5%) and glucose (13.5%).

12/7/52

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14370170 BIOSIS NO.: 199800164417

Purification and properties of inulinase from *Aspergillus niger*

AUTHOR: Chen Guanjin; Sun Zhongdong; Wang Yingda; Qian Xinmin

AUTHOR ADDRESS: State Key Lab. Microbial Technol., Shandong Univ., Jinan 250100, China**China

JOURNAL: Weishengwu Xuebao 37 (5): p362-367 Oct., 1997 1997

MEDIUM: print

ISSN: 0001-6209

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: Chinese

ABSTRACT: The main component of inulinase was purified from fermentation broth of *Aspergillus niger* 319 to homogeneity by using ammonium sulfate fraction, ion-exchange chromatography on DEAE-cellulose column and Sephadex G-100 gel filtration. The specific activity was as 67 folds at the fermentation broth, and the yield was 25.5%. The inulinase, containing 13.92% of carbohydrate, was a monomer protein with a molecular weight of 28000 Dalton; and its isoelectric point was pH 5.4. The optimal pH and temperature of the inulinase was pH 5.0 and 60 degree C, respectively. The enzyme was strongly inhibited by heavy metal ions of Hg²⁺, Pb²⁺ and Cu²⁺. The optimal substrate for the enzyme was inulin and the product was only fructose, but it also had invertase activity with the I/S of 0.348. The K_m and V_m of the inulinase was 6.25 mmol L and 67.11 μmol cntdot mg⁻¹ cntdot min⁻¹, respectively.

12/7/53

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14015194 BIOSIS NO.: 199799649254

Purification and substrate specificity of an extracellular fructan hydrolase from *Lactobacillus paracasei* ssp. *paracasei* P 4134

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JOURNAL: New Phytologist 136 (1): p89-96 1997 1997

ISSN: 0028-646X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A novel extracellular fructanhydrolase was isolated from the culture filtrate of *Lactobacillus paracasei* ssp. *paracasei* P 4134 grown

on a mineral medium supplemented with fructan extracted from Timothy (*Phleum pratense* L.) as the only carbon source. The enzyme was **purified** by a combination of ammonium sulphate precipitation, affinity chromatography, preparative isoelectric focusing and anion-exchange chromatography. As a result of these procedures, the specific enzyme activity increased 93-fold, with a final yield of 28-4%. The substrate-specific activities against different fructan types were determined by incubating the enzyme fractions with fructan extracted from Timothy (predominantly beta-2,6 fructosyl-fructose linkages), **inulin** from Dahlia tubers (mostly beta-2,1 fructosyl-fructose linkages) and sucrose. The **purified** enzyme catalysed the hydrolysis of beta-2,6-linked fructan more rapidly than the beta-2,1 linkages of **inulin**. Additionally, the enzyme showed low ability to hydrolyse sucrose. Fructose was the main product of the degradation of Timothy fructan and **inulin**, indicating a high exohydrolytic activity of the enzyme. It is proposed that the fructan-degrading enzyme from *L. paracasei* ssp. *paracasei* P 4134 is a beta-D-fructan-fructohydrolase (EC 3.2.1.80). The enzyme preparation showed a single **protein** band in sodium dodecyl sulphate-polyacrylamide gel electrophoresis with a mobility corresponding to molecular weight of c. 42 kDa. It was concluded that only one molecular weight of fructan-degrading enzyme exists in *L. paracasei* ssp. *paracasei* P 4134.

12/7/54

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13889694 BIOSIS NO.: 199799523754

Seasonal variation of fructan-beta-fructosidase (FEH) activity and characterization of a beta-(2-1)-linkage specific FEH from tubers of Jerusalem artichoke (*Helianthus tuberosus*)

AUTHOR: Marx Stefan P; Nosberger Josef; Frehner Marco (Reprint)

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JOURNAL: New Phytologist 135 (2): p267-277 1997 1997

ISSN: 0028-646X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The fructan-beta-fructosidase activity (1-FEH; EC 3. 2.1. 80) that degrades **inulin** in tubers of *Helianthus tuberosus* L. appears to be developmentally regulated; it was low in growing tubers but increased during dormancy and sprouting. In spite of relatively high 1-FEH activity in vitro, fructose concentration was very low in developing and dormant tubers and increased markedly only during sprouting. A fructan-beta-fructosidase from such sprouting tubers was **purified** 41 -fold to a single **protein** band on one-dimensional sodium dodecylsulphate-polyacrylamide gels. The **purification** procedure included ammonium sulphate precipitation, lectin-affinity chromatography on concanavalin A, anion-exchange and cation-exchange chromatography. The enzyme had an apparent molecular mass of 75000 measured by size-exclusion chromatography, and 79000 measured by one-dimensional sodium dodecylsulphate-polyacrylamide gel electrophoresis. It exhibited a high substrate specificity, hydrolysing terminal beta-(2-1)-fructosyl-fructose-linkages in linear and branched

fructan oligomers; beta-(2-6)-linkages were hardly hydrolysed. Hydrolysis of *inulin* oligomers followed normal saturation kinetics: K_m values for 1,1-kestotetraose and 1,1,1-kestopentaose were 8-3 mM and 12 mM, respectively. Fructosyl residues were hydrolysed from *inulin* oligomers by a multi-chain mechanism. The fructan-beta-fructosidase showed optimal enzyme activity at pH 5-2, and it retained its full activity after pre-incubation for 1 h at up to 40 degree C. The release of fructose from 5 mM 1,1-kestotetraose was reduced by 25% when 1-FEH was assayed in the presence of 10 mM sucrose. It is proposed that the inhibition of 1-FEH activity by sucrose is a mechanism for controlling fructan degradation in planta.

12/7/55

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13571525 BIOSIS NO.: 199699205585

Purification and characterization of fructan: Fructan fructosyl transferase from chicory (*Cichorium intybus* L.) roots

AUTHOR: Van Den Ende Wim; Van Wonterghem Dominik; Verhaert Peter; Dewil Erna; Van Laere Andre (Reprint)

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JOURNAL: Planta (Heidelberg) 199 (4): p493-502 1996 1996

ISSN: 0032-0935

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Fructan: fructan fructosyl transferase (FFT, EC 2.4.1.100) was *purified* from chicory (*Cichorium intybus* L. var. foliosum cv. Flash) roots by a combination of ammonium sulfate precipitation, concanavalin A affinity chromatography, and anion- and cation-exchange chromatography. This protocol produced a 60-fold *purification* and a specific activity of 14.5 μ -mol cntdot (mg *protein*)-1 cntdot min-1. The mass of the enzyme was 69 kDa as estimated by gel filtration. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis and mass spectrometry, 52-kDa and 17-kDa fragments were found, suggesting that the enzyme was a heterodimer. Optimal activity was found between pH 5.5 and 6.5. The enzyme used 1-kestose, 1,1-nystose, oligofructan and commercial chicory root *inulin* (degree of polymerization ≥ 10) as donors and acceptors. Sucrose was the best acceptor but could not be used as a donor. However, at higher concentrations sucrose acted as a competitive inhibitor for donors of FFT. 1-Kestose was the most efficient and 1,1-nystose the least efficient donor. The *purified* enzyme exhibited beta-fructosidase activity, specially at higher temperatures and lower substrate concentrations. The synthesis of fructans from 1-kestose decreased at higher temperatures (5-50 degree C). Therefore enzyme assays were performed at 0 degree C. The same fructan oligosaccharides, with a distribution similar to that observed in vivo, were obtained upon incubation of the enzyme with sucrose and commercial chicory root *inulin*.

12/7/56

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13317004 BIOSIS NO.: 199698784837

Cross-linked hemoglobin increases fractional reabsorption and GFR in hypoxic isolated perfused rat kidneys

AUTHOR: Baines A D (Reprint); Christoff B; Wicks D; Wiffen D; Pliura D

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JOURNAL: American Journal of Physiology 269 (5 PART 2): pF628-F636 1995 1995

ISSN: 0002-9513

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: We compared the ability of human red blood cells (RBC) and a cell-free oxygen carrier to maintain isolated perfused kidney function under moderately hypoxic conditions. Recirculating perfusate was gassed initially with 93% air-7% CO-2, and, after 30 min, the gas was changed to 12 O-2-7 CO-2-81% N-2. Oxygen content of the perfusate was increased with RBC (30 g/l Hbg) or highly %purified% human hemoglobin A-0 (HbA-0) polymerized with O-raffinose (o-R-poly-Hb, 30 g/l Hbg). For comparison, kidneys were perfused with 60 g/l of bovine serum albumin (BSA) alone. The effects of unmodified hemoglobin were examined by adding 5 g/l of nonpolymerized HbA-0 to the BSA perfusate after 20 min. The effect of increasing oxygen delivery without hemoglobin was examined by switching to 93% O-2 after 20 min during some BSA perfusions (BSA-HiO-2). Vascular resistance decreased progressively in o-R-poly-Hb- and BSA-HiO-2-perfused kidneys but remained constant in other experiments. Nitro-L-arginine methyl ester (L-NAME) prevented vasodilation and increased the filtration fraction of o-R-poly-Hb-perfused kidneys with no change in other functions. L-NAME also prevented the formation of methemoglobin. After a 70-min perfusion with BSA, Na reabsorption was $82 \pm 3\%$ (means \pm SD), and %inulin% clearance (glomerular filtration rate (GFR)) was 0.66 ± 0.33 ml cntdot min-1 cntdot g-1. RBC increased reabsorption to 95% (85-98%) (median, 25th-75th percentile) but did not alter GFR (0.52 ± 0.26 ml cntdot min-1 cntdot g-1). o-R-poly-Hb increased Na reabsorption proportionately more than GFR, so that, while GFR was doubled to 1.04 ± 0.40 ml cntdot min-1 cntdot g-1, Na reabsorption increased to 98% (92-99.5%). HbA-0 increased GFR to 1.07 ± 0.44 ml cntdot min-1 cntdot g-1 and increased reabsorption to $89 \pm 6\%$. A similar increase in Na reabsorption ($93 \pm 2\%$) and GFR (1.38 ± 0.3 ml cntdot min-1 cntdot g-1) was produced by increasing O-2 content of BSA with 93% O-2. o-R-poly-Hb was most effective in raising and maintaining overall renal function and lowering urine Na concentration and %protein% excretion.

12/7/57

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13037659 BIOSIS NO.: 199598505492

%%Purification%% and characterization of the invertase from Pycnoporus sanguineus

AUTHOR: Quiroga Emma Nelly; Vattuone Marta Amelia; Sampietro Antonio Rodolfo

AUTHOR ADDRESS: Catedra Fitoquim., Inst. Estudios Vegetales, Fac. Bioquim.,

Quim. Farm., Univ. Nacl. Tucuman, Ayacucho 461, 4000-San Miguel Tucuman,
Argentina**Argentina
JOURNAL: Biochimica et Biophysica Acta 1251 (2): p75-80 1995 1995
ISSN: 0006-3002
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A constitutive invertase (EC 3.2.1.26) was isolated and
%%purified%% by the first time from Pycnopus sanguineus. The enzyme
is a glycoprotein. Its relative molecular mass is about 84 000 and its
structure is dimeric, with two identical subunits (about 41 000). The
enzyme is able to attack sucrose, raffinose, stachyose, %%inulin%% and
levan, being sucrose the preferred substrate (K_m 4.89 \pm 0.13 mM).
Fructose was a classical competitive inhibitor, but glucose was not an
inhibitor of the enzyme. Lectins with specificity toward glucose are
inhibitors of the enzyme. Glucose was present in invertase acid
hydrolysates. Unlike higher plant invertases, bovine serum albumin is not
an effector of the Pycnopus sanguineus enzyme, and the inhibition by
fructose is not suppressed by this %%protein%%. The properties of the
Pycnopus sanguineus enzyme are discussed and reference to higher plant
invertases.

12/7/58
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12999013 BIOSIS NO.: 199598466846
Continuous production of fructose syrups from %%inulin%% by immobilized
inulinase from Aspergillus niger mutant 817
AUTHOR: Nakamura Toyohiko; Ogata Yasuko; Shitara Akichika; Nakamura Akihiro
; Ohta Kazuyoshi (Reprint)
AUTHOR ADDRESS: Dep. Biol. Resource Sci., Fac. Agric., Miyazaki Univ., 1-1
Gakuen Kibanadai Nishi, Miyazaki 889-21, Japan**Japan
JOURNAL: Journal of Fermentation and Bioengineering 80 (2): p164-169 1995
1995
ISSN: 0922-338X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Aspergillus niger mutant 817 was grown in submerged culture with
sucrose. Inulinase was partially %%purified%% from the culture filtrate
by DEAE-Cellulofine A-500 chromatography. The complex enzyme preparation
containing both exo- and endoinulinases was immobilized covalently onto a
porous cellulose derivative, Amino-Cellulofine, by the carbodiimide
method at pH 5.0. The immobilized enzyme had 160 U inulinase activity/g
(wet wt.) of the support, with the immobilization yield of 96% on a
%%protein%% basis and the activity yield of 15%. The maximum inulinase
activity occurred at pH 5.2 and 50 degree C. The immobilized enzyme was
stable in the pH ranges of 4.5 to 6.5 at 30 degree C and 5.0 to 6.0 at 50
degree C. Enzyme stability was retained up to 60 degree C. In a
packed-bed column reactor containing 8 ml of the immobilized inulinase, a
5.0% (w/v) solution (pH 5.0) of pure dahlia %%inulin%% was completely
hydrolyzed at a flow rate of 1.0 ml/min at 40 degree C over a 45-d period
of continuous operation. The volumetric productivity in the reactor was

410 g reducing sugars/l/h. The reaction product was a mixture of 97% D-fructose and 3% D-glucose.

12/7/59

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12812164 BIOSIS NO.: 199598279997

%%Purification%% and characterization of the Bacillus subtilis levanase produced in Escherichia coli

AUTHOR: Wanker Erich; Huber Anton; Schwab Helmut (Reprint)

AUTHOR ADDRESS: Inst. Biotechnol., Arbeitsgruppe Genetik, Technische Univ., Petergasse 12, A-8010 Graz, Austria**Austria

JOURNAL: Applied and Environmental Microbiology 61 (5): p1953-1958 1995 1995

ISSN: 0099-2240

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The enzyme levanase encoded by the sacC gene from Bacillus subtilis was overexpressed in Escherichia coli with the strong, inducible tac promoter. The enzyme was %purified% from crude E. coli cell lysates by salting out with ammonium sulfate and chromatography on DEAE-Sephadex CL-6B, S-Sephadex, and MonoQ-Sephadex. The %purified% %protein% had an apparent molecular mass of 75,000 Da in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, which is in agreement with that expected from the nucleotide sequence. Levanase was active on levan, %inulin%, and sucrose with K-m values of 1.2 mu-M, 6.8 mM, and 65 mM, respectively. The pH optimum of the enzyme acting on %inulin% was 5.5, and the temperature optimum was 55 degree C. Levanase was rapidly inactivated at 60 degree C, but activity could be retained for longer times by adding fructose or glycerol. The enzyme activity was completely inactivated by Ag+ and Hg-2+ ions, indicating that a sulfhydryl group is involved. A ratio of sucrase to inulinase activity of 1.2 was found for the %purified% enzyme with substrate concentrations of 50 mg/ml. The mechanism of enzyme action was investigated. No liberation of fructo-oligomers from %inulin% and levan could be observed by thin-layer chromatography and size exclusion chromatography-low-angle laser light scattering-interferometric differential refractive index techniques. This indicates that levanase is an exoenzyme acting by the single-chain mode.

? s fructan and hemoglobin

1155 FRUCTAN

89346 HEMOGLOBIN

S14 1 FRUCTAN AND HEMOGLOBIN

? t s14/7/1

14/7/1

DIALOG(R)File 5:Biosis Previews(R)

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0020382872 BIOSIS NO.: 200800429811

Effects of supplemental inulin on utilization of iron in corn-soy diet by young pigs for %hemoglobin% synthesis

AUTHOR: Yasuda K (Reprint); Roneker K R; Miller D D; Welch R M; Lei X G

AUTHOR ADDRESS: Cornell Univ, Ithaca, NY USA**USA
 JOURNAL: Journal of Dairy Science 88 (Suppl. 1): p31 2005 2005
 CONFERENCE/MEETING: Annual Meeting of the
 American-Dairy-Science-Association/American-Society-of-Animal-Science/Canadian-Society-of-Animal-Science Cincinnati, OH, USA July 24 -28, 2005;
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 Canadian Soc Animal Sci
 ISSN: 0022-0302
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 LANGUAGE: English
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Set	Items	Description
S1	9	INULIN AND (REDUCING() SUGAR)
S2	5	INULIN AND TAGATOSE
S3	992	INULIN AND GLUCOSE
S4	122	INULIN AND (FREEZE OR LYOPHIL? OR AIR)
S5	48	INULIN AND HEMOGLOBIN
S6	8	S5 AND GLUCOSE
S7	0	S5 AND TAGATOSE
S8	0	INULIN AND (PEG?(3)HEMOGLOBIN)
S9	0	INULIN AND (PEG?(3W)HEMOGLOBIN)
S10	0	S1 AND S4
S11	2	S4 AND S5
S12	88	INULIN AND (PURIF? AND PROTEIN)
S13	12	S3 AND (PURIF? AND PROTEIN)
S14	1	FRUCTAN AND HEMOGLOBIN

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